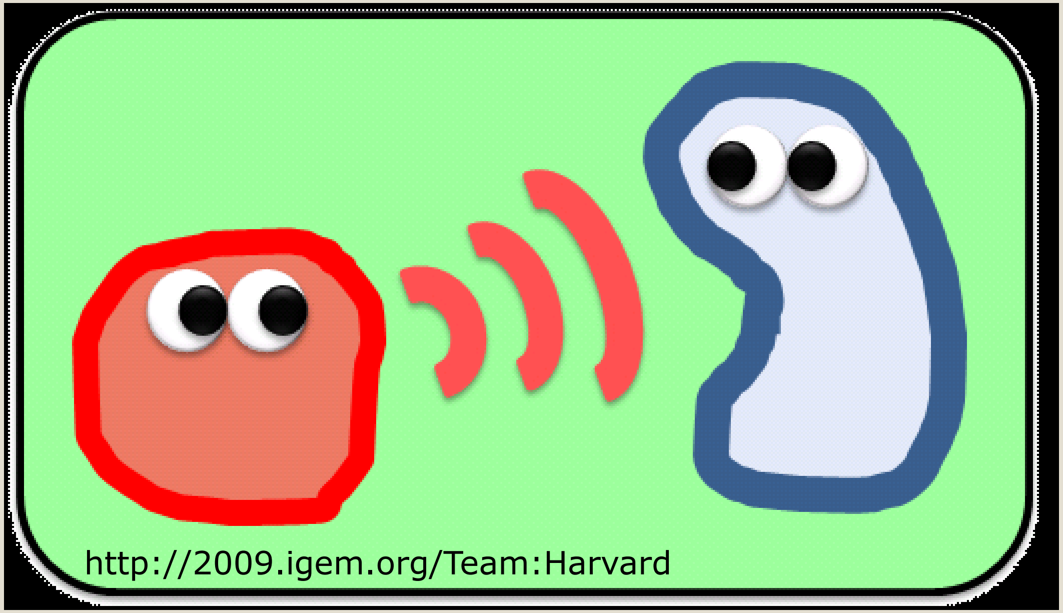
Cell-to-cell communication



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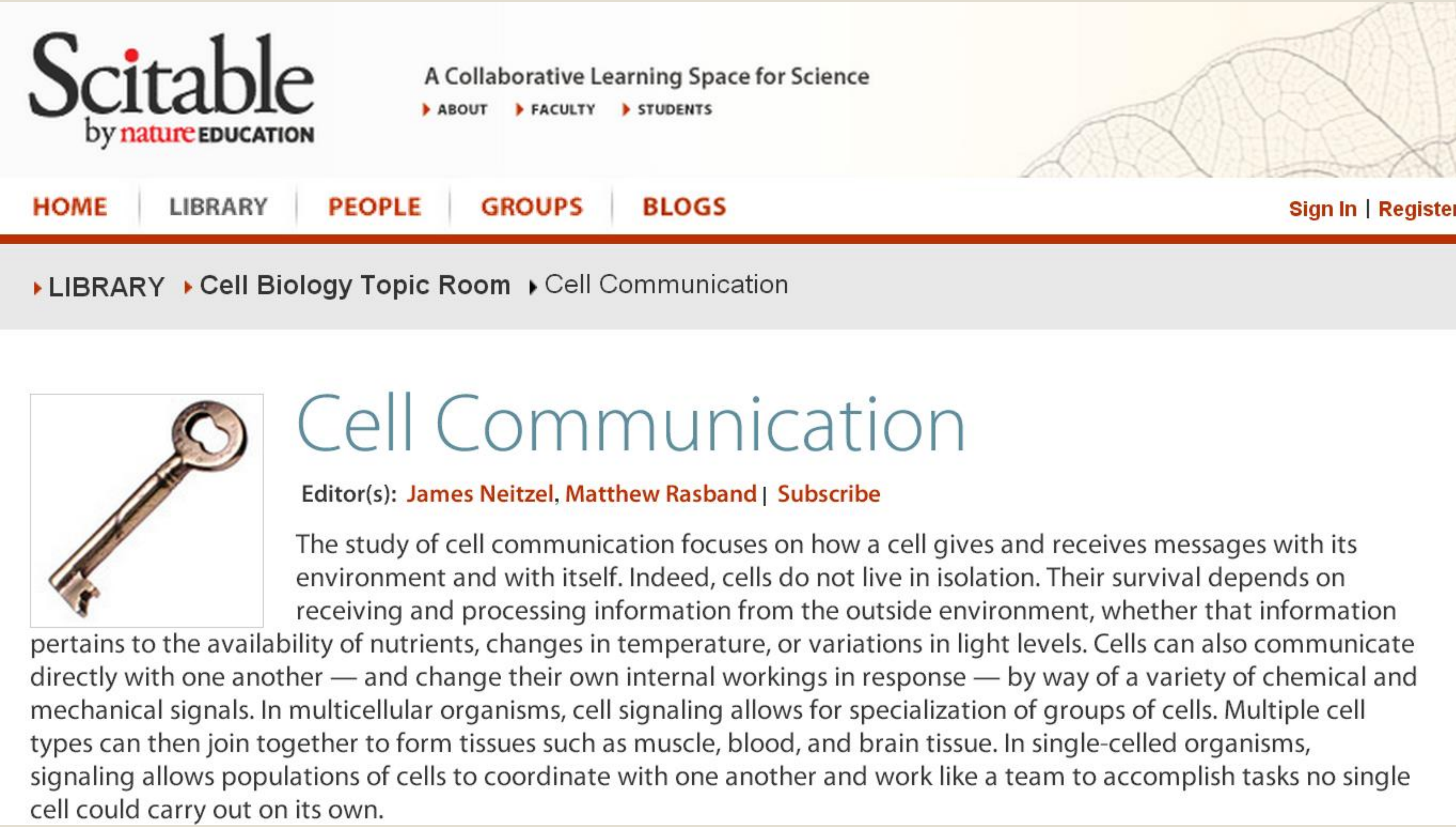
Cell-to-cell communication: molecular mechanisms

Lecture 1

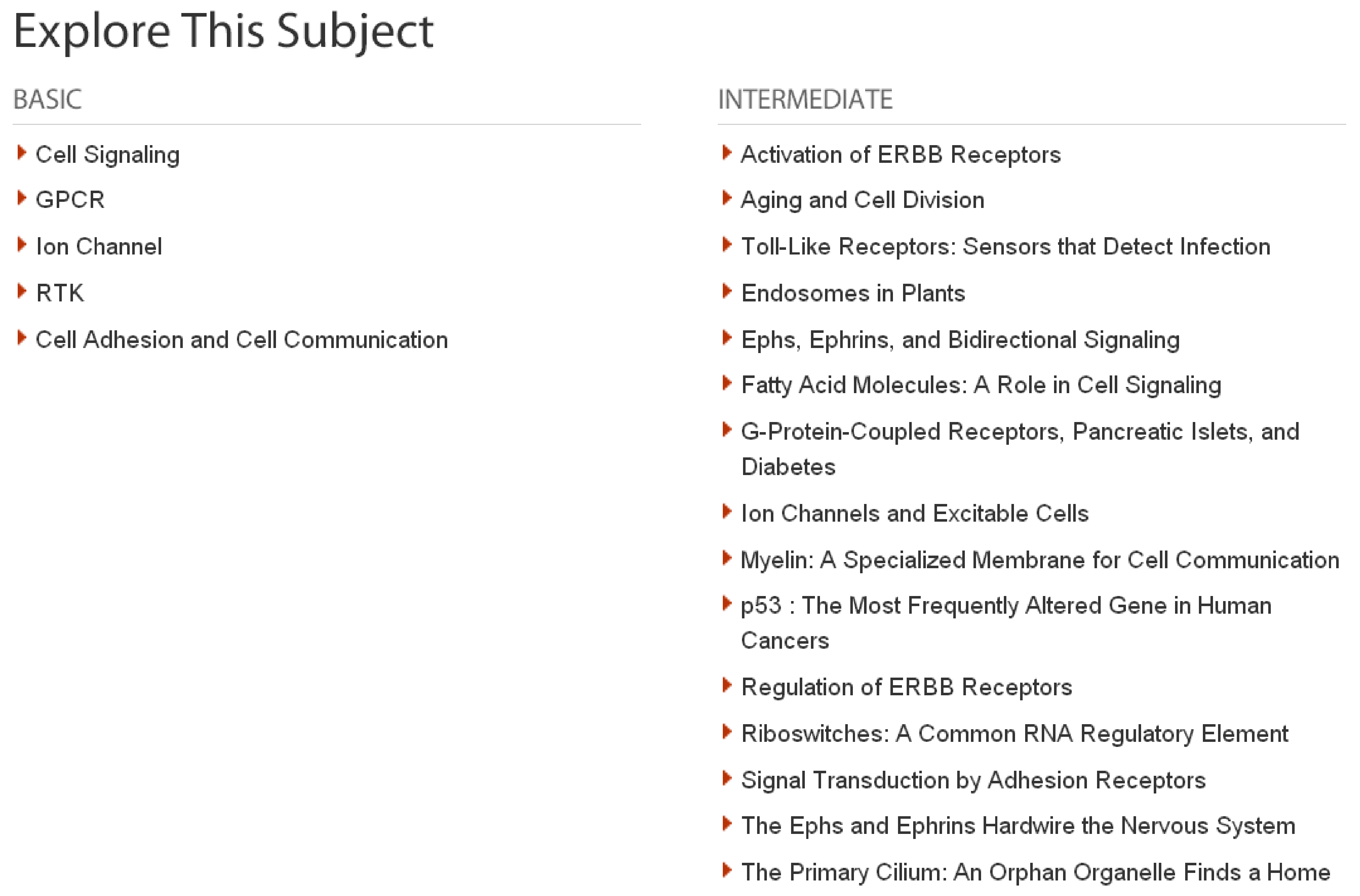


Cell Communication

Editor(s): James Neitzel, Matthew Rasband | Subscribe

The study of cell communication focuses on how a cell gives and receives messages with its environment and with itself. Indeed, cells do not live in isolation. Their survival depends on receiving and processing information from the outside environment, whether that information pertains to the availability of nutrients, changes in temperature, or variations in light levels. Cells can also communicate directly with one another — and change their own internal workings in response — by way of a variety of chemical and mechanical signals. In multicellular organisms, cell signaling allows for specialization of groups of cells. Multiple cell types can then join together to form tissues such as muscle, blood, and brain tissue. In single-celled organisms, signaling allows populations of cells to coordinate with one another and work like a team to accomplish tasks no single cell could carry out on its own.

http://www.nature.com/scitable/topic/cell-communication-14122659



Explore This Subject

BASIC

- Cell Signaling
- GPCR
- Ion Channel
- RTK
- Cell Adhesion and Cell Communication

INTERMEDIATE

- Activation of ERBB Receptors
- Aging and Cell Division
- Toll-Like Receptors: Sensors that Detect Infection
- Endosomes in Plants
- Ephs, Ephrins, and Bidirectional Signaling
- Fatty Acid Molecules: A Role in Cell Signaling
- G-Protein-Coupled Receptors, Pancreatic Islets, and Diabetes
- Ion Channels and Excitable Cells
- Myelin: A Specialized Membrane for Cell Communication
- p53 : The Most Frequently Altered Gene in Human Cancers
- Regulation of ERBB Receptors
- Riboswitches: A Common RNA Regulatory Element
- Signal Transduction by Adhesion Receptors
- The Ephs and Ephrins Hardwire the Nervous System
- The Primary Cilium: An Orphan Organelle Finds a Home

Cell (cell-to-cell) communication

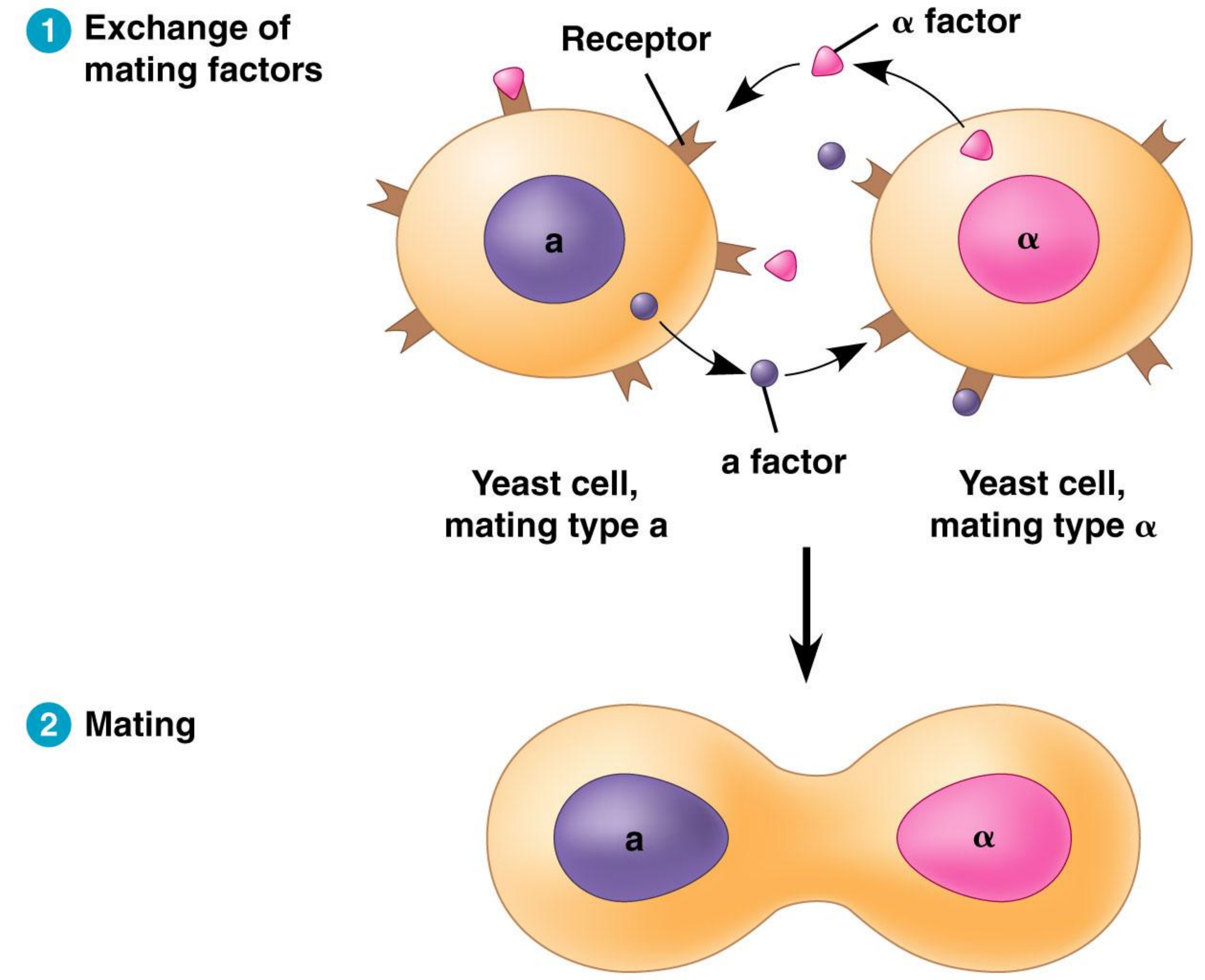
Cellular communication is term used in biophysics and biochemistry to identify different types of communication methods between living cells. **Some of the methods include cell signaling among others**.

“Cell Signaling”

Cell signalling (**Cell signaling** in American English) is part of a complex system of communication that governs basic cellular activities and coordinates cell actions. The ability of cells to perceive and correctly respond to their microenvironment **is the basis of development, tissue repair, and immunity as well as normal tissue homeostasis**.

- Cell signaling has been most extensively studied in the context of human diseases and signaling between cells of a single organism.
- However, **cell signaling may also occur between the cells of two different organisms**.
- Single celled organisms **use intercellular signals** to coordinate such things as gene expression, mating, sporulation and cell death in response to population density, nutrients, stress and other cues**.**

Signalling, Free-Living Cells


1 Exchange of mating factors
Receptor
α factor
a
α
a factor
Yeast cell, mating type a
Yeast cell, mating type α
2 Mating
a
α

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Multicellular organisms use intercellular communications to coordinate the activities of their component cells**.** The overall purpose is to coordinate the activities of multiple cells in response to the needs of the organism and changes in its environment.

- We have evolved very complex cell communications systems to regulate our **100 trillion cells**. These pathways are similar to and likely arose from those that single celled organisms use to molecularly sense their environments.
- **Much of our genetic energy is spent on cell signaling and control**.

Types of cell signaling in multicellular organisms

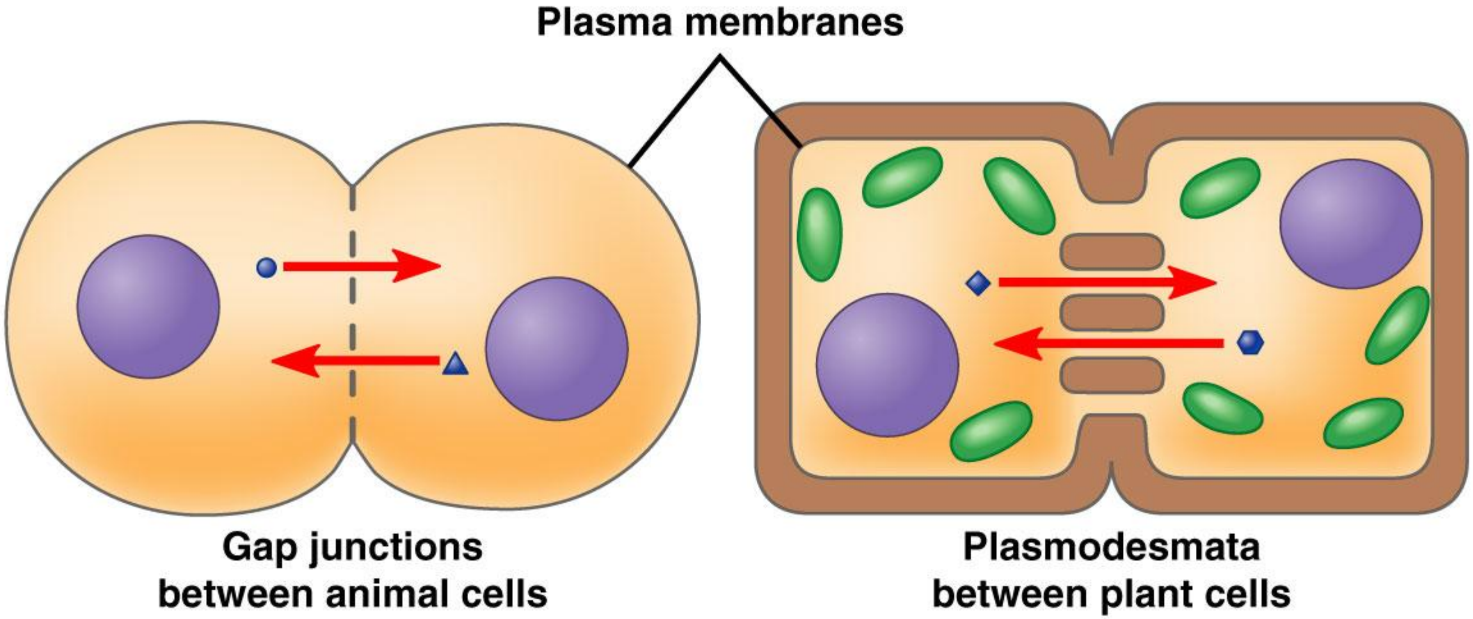
- Cells can signal to themselves (usually to become more active)
- Cell-cell communication within a tissue
- Paracrine signaling- signals are labile; inflammation is an example
- Endocrine- hormones: delivered through bloodstream; more stable
- Synaptic- neurotransmitters. Highly localized

Chemical Signaling Between Cells

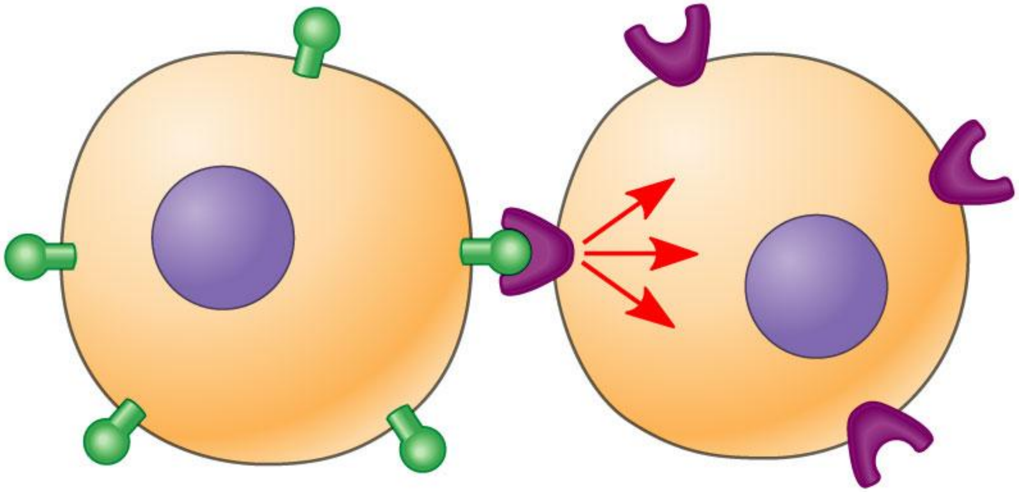
❑ **2 GENERAL CATEGORIES** of chemical signaling:

- Cell-to-cell **contact-mediated** signaling (includes diffusion of signal molecules inside cells linked by cytoplasmic connections)
- Free **diffusion of signal molecules between cells**
  - Distant cells (hormones)
  - Adjacent cells (within interstitial space – paracrine, autocrine regulation)
  - Between single-celled organisms (including bacteria)
  - Between single-celled and multicellular organisms

Signaling with Direct Contact


Plasma membranes
Gap junctions between animal cells
Plasmodesmata between plant cells

(a) Cell junctions



(b) Cell-cell recognition

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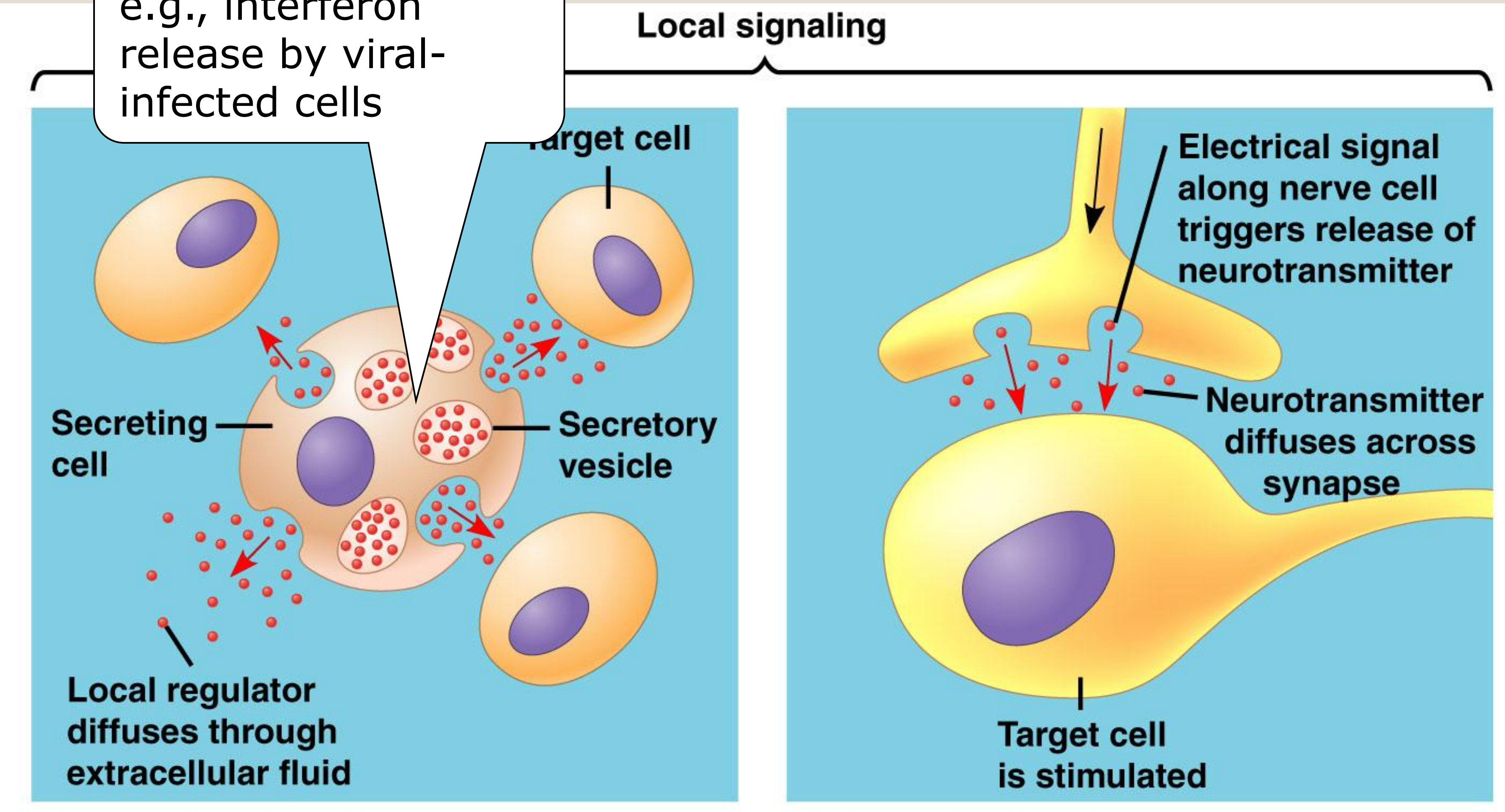
Local Signaling w/o Direct Contact

e.g., interferon release by viral-infected cells


Local signaling
arget cell
Secreting cell
Secretory vesicle
Local regulator diffuses through extracellular fluid
Electrical signal along nerve cell triggers release of neurotransmitter
Neurotransmitter diffuses across synapse
Target cell is stimulated

(a) Paracrine signaling (b) Synaptic signaling

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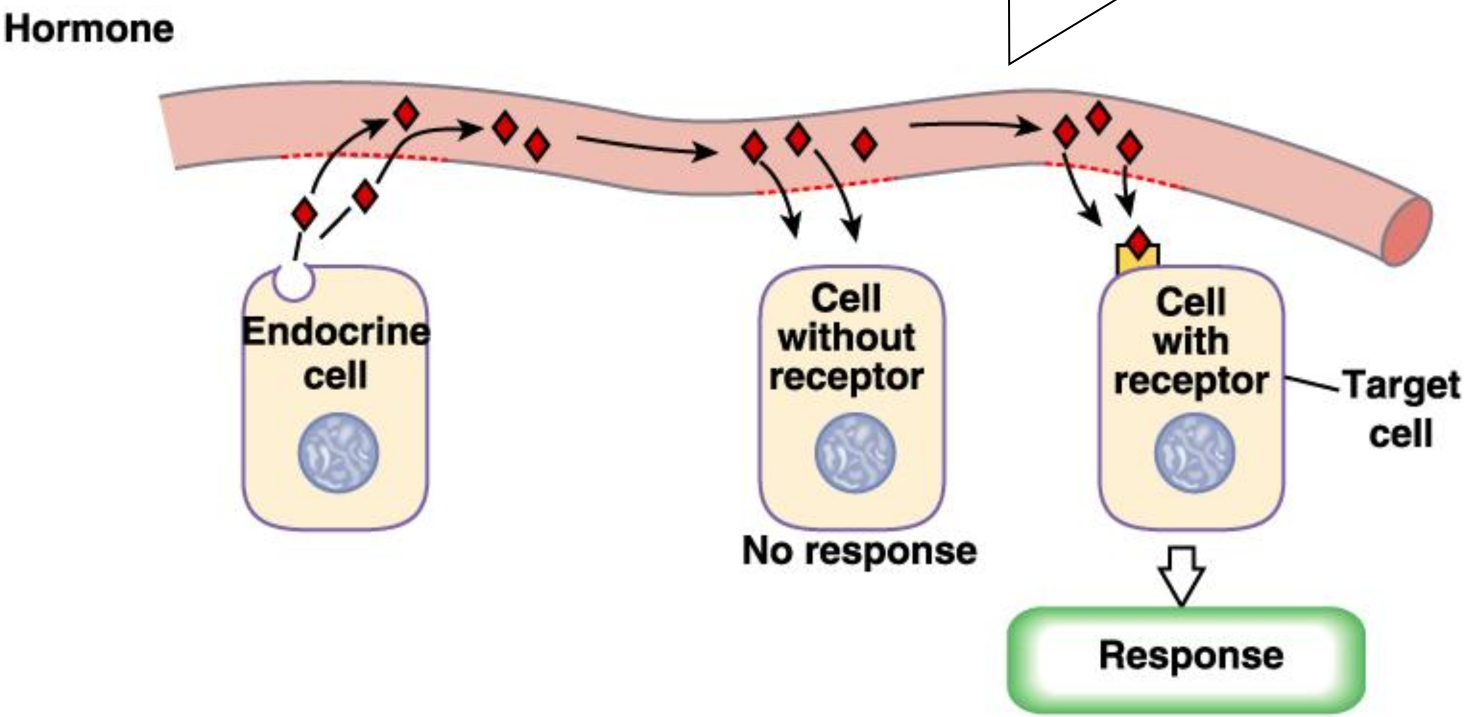
Long-Distance Diffusion

Note how specificity is determined by presence/absence of receptor protein



What do we mean by “cell signaling”?

- The cell “signal” is a small molecule (can be a gas)
- It binds to a specific receptor on the target cell
- Response mechanisms are activated in the target cell
- Response often involves gene transcription

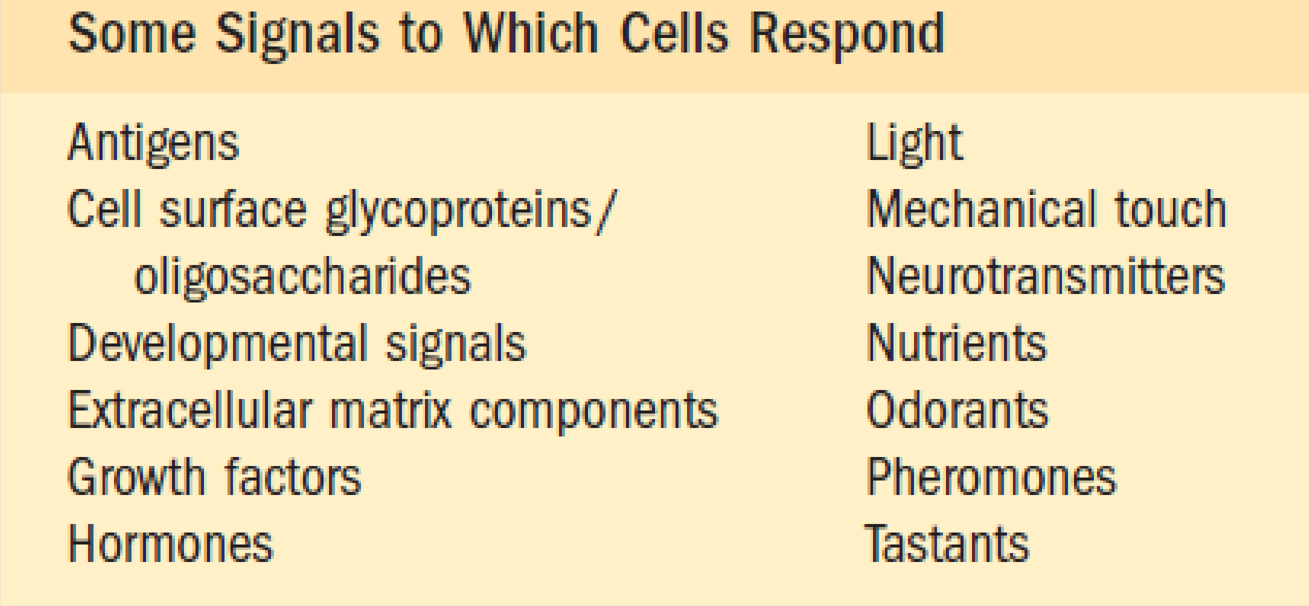
Signaling and Signal transduction

Different signals elicit appropriate responses, such as motion toward food or away from toxic substances or the formation of dormant spores in a nutrient-depleted medium.

In all cases, **the signal represents information that is detected by specific receptors and converted to a cellular response**, which always involves a chemical process.

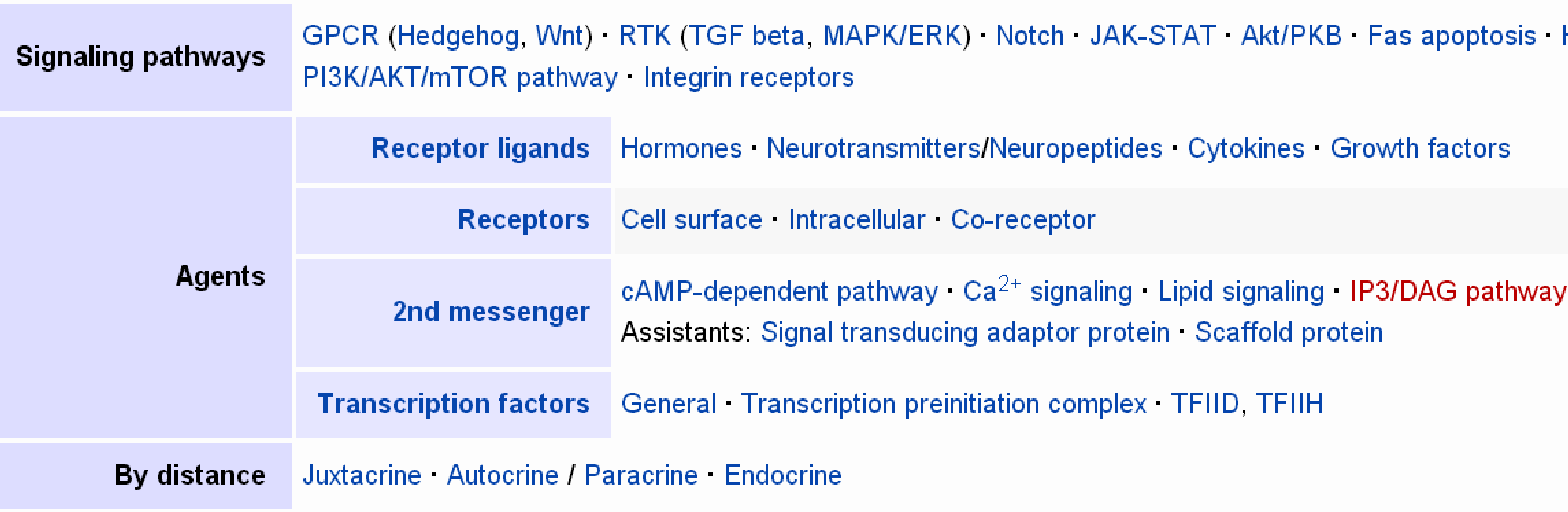
This **conversion of information into a chemical change, *signal transduction*,** is a universal property of living cells.

The number of different biological signals is large, as is **the variety of biological responses** to these signals, but organisms use just **a few evolutionarily conserved mechanisms to detect extracellular signals and transduce them into intracellular changes**.



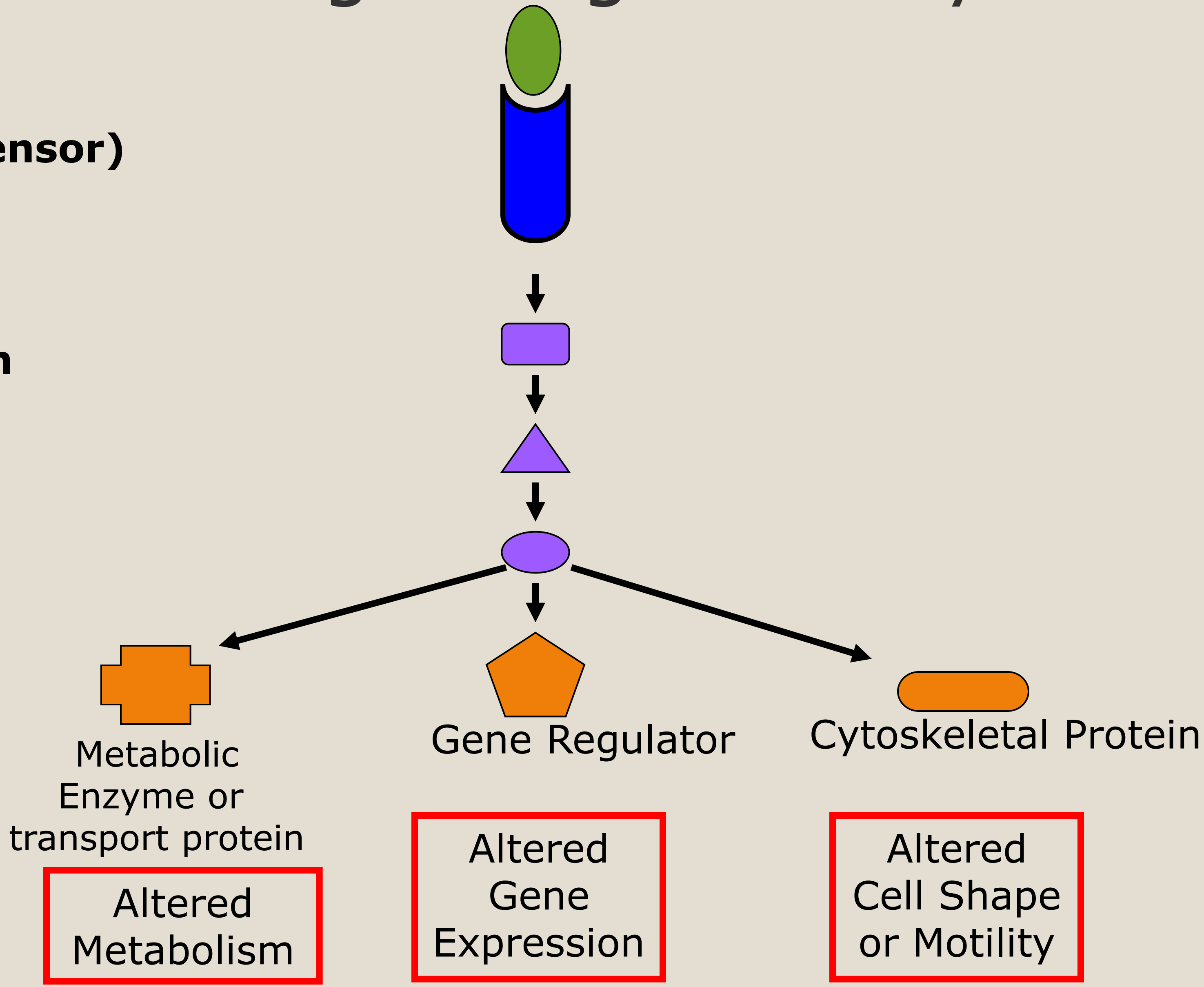
Some Signals to Which Cells Respond

- Antigens
- Cell surface glycoproteins/oligosaccharides
- Developmental signals
- Extracellular matrix components
- Growth factors
- Hormones
- Light
- Mechanical touch
- Neurotransmitters
- Nutrients
- Odorants
- Pheromones
- Tastants



Signaling pathways	GPCR (Hedgehog, Wnt) · RTK (TGF beta, MAPK/ERK) · Notch · JAK-STAT · Akt/PKB · Fas apoptosis · PI3K/AKT/mTOR pathway · Integrin receptors	
Agents	**Receptor ligands**	Hormones · Neurotransmitters/Neuropeptides · Cytokines · Growth factors
	Receptors	Cell surface · Intracellular · Co-receptor
	2nd messenger	cAMP-dependent pathway · Ca^{2+} signaling · Lipid signaling · IP3/DAG pathway Assistants: Signal transducing adaptor protein · Scaffold protein
	Transcription factors	General · Transcription preinitiation complex · TFIID, TFIIH
By distance	Juxtacrine · Autocrine / Paracrine · Endocrine	

Generic Signalling Pathway



Signal

Receptor (sensor)

Transduction Cascade

Targets

Metabolic Enzyme or transport protein

Gene Regulator

Cytoskeletal Protein

Response

Altered Metabolism

Altered Gene Expression

Altered Cell Shape or Motility

Adapted from Molecular Biology of the Cell,(2002), 4th edition, Alberts et al.

Parameters of signal transduction

1. **Signals (physical; chemical) affecting receptors;** properties of molecules(*hydrophobicity*), their transport → *location of R* (surface/ intracellular)
2. **Properties of receptors** (R): specificity to signal (type of signal → type of R); ability to transduce the signal (binding/ catalytic); adaptation of R to signal (desensitization; feedback regulation)
3. **Signaling pathway**: structure of adaptors; amplification of signal (2 strategies depending on surface/intracellular location of R); integration of signal pathways (cross-talk; coordination of response)
4. **Molecular targets and time necessary for response** (quick/prolonged depending on protein synthesis)
5. For multicellular organisms: **hierarchy of signal systems**; location-, dose- and time-dependent effects

2 types of signaling molecules

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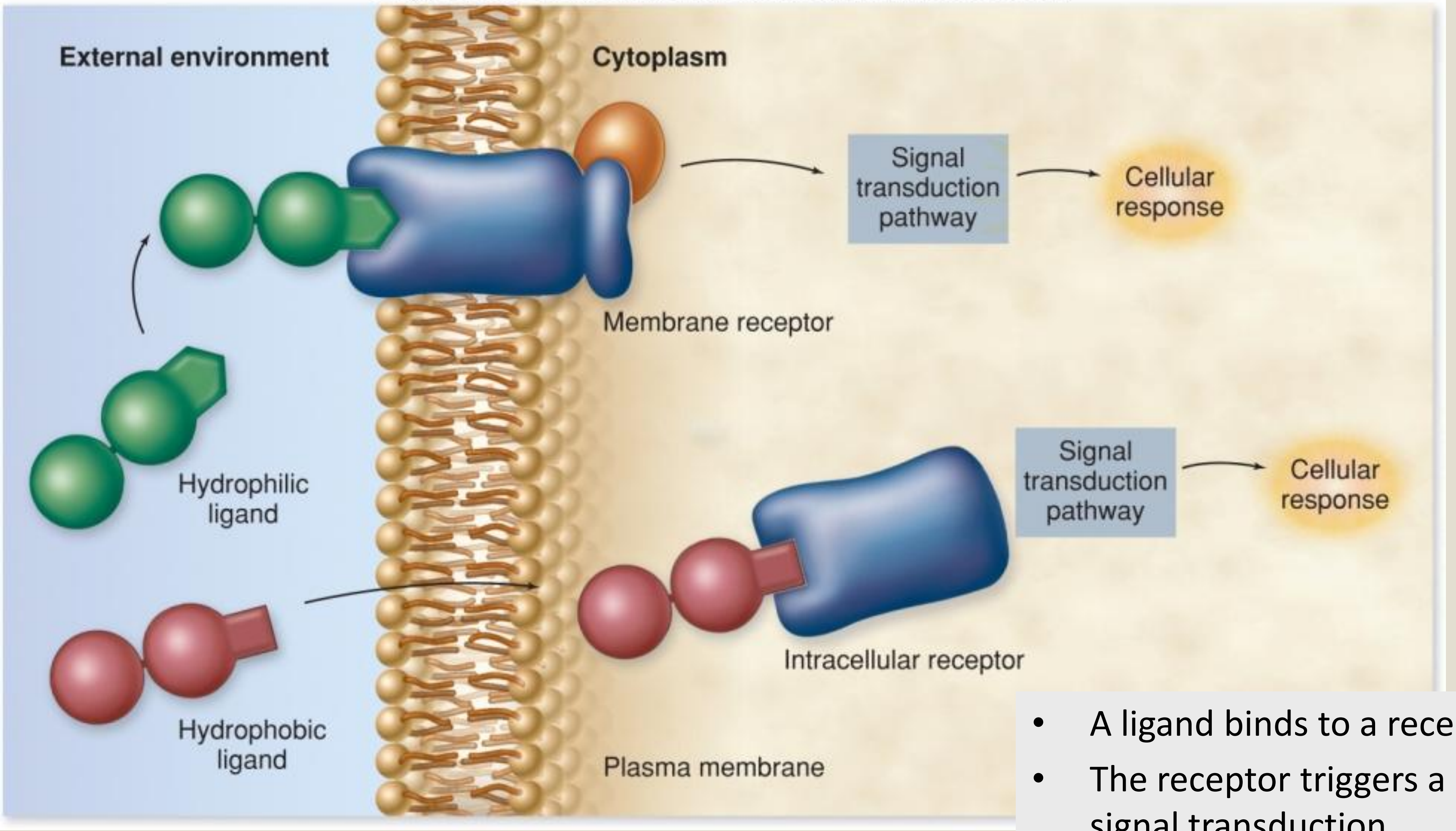
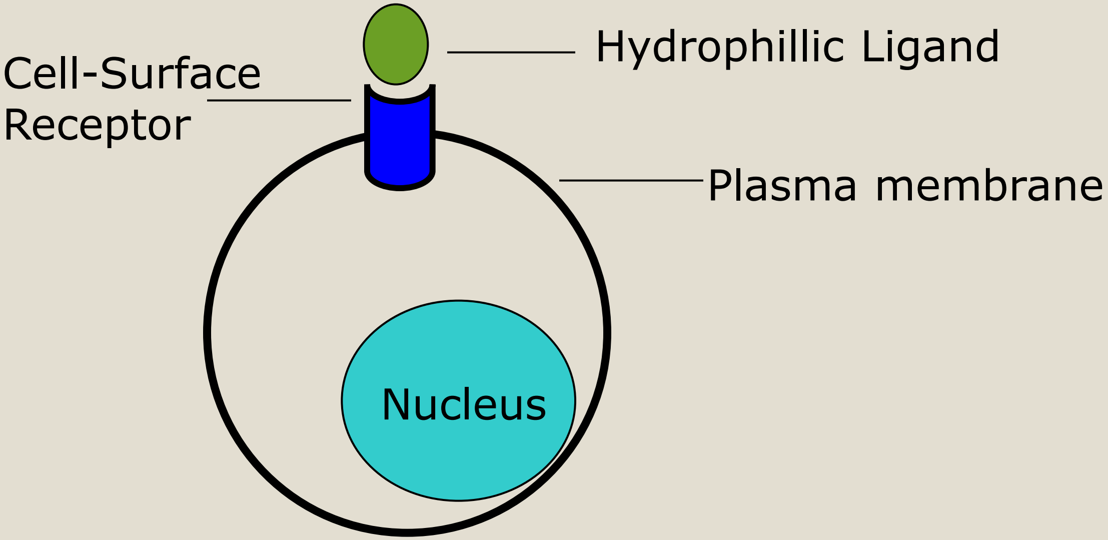


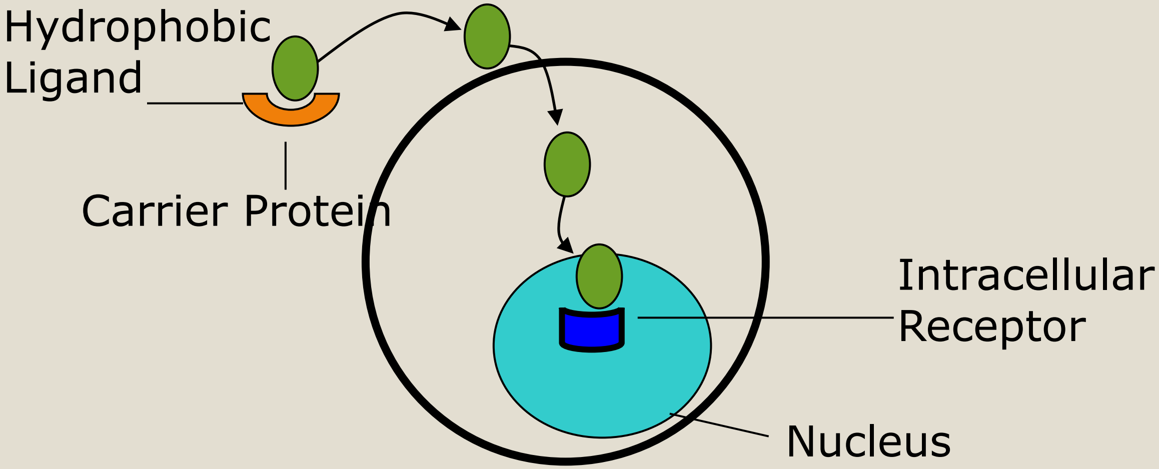
- A ligand binds to a receptor
- The receptor triggers a signal transduction
- This causes a change in the cell

Hydrophobicity of signal→ location of R

Cell surface



Intracellular



Adapted from Molecular Biology of the Cell,(2002), 4th edition, Alberts et al.

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Where are the receptors?

- Inside the cell
  - can bind hydrophobic ligands (often lipid)
- In the cell membrane
  - Ligand is hydrophilic; does not cross membrane
  - Transmembrane protein is changed when ligand binds
  - The receptor delivers the signal

“Superfamilies” of receptors

- Have characteristic “domains” (functional parts of proteins)
  - Ligand binding
  - DNA binding
  - Binding to regulatory molecules
- Activity is regulated within the cell
  - Coactivators
  - Coactivator levels may vary among tissues

Intracellular receptors affect gene transcription

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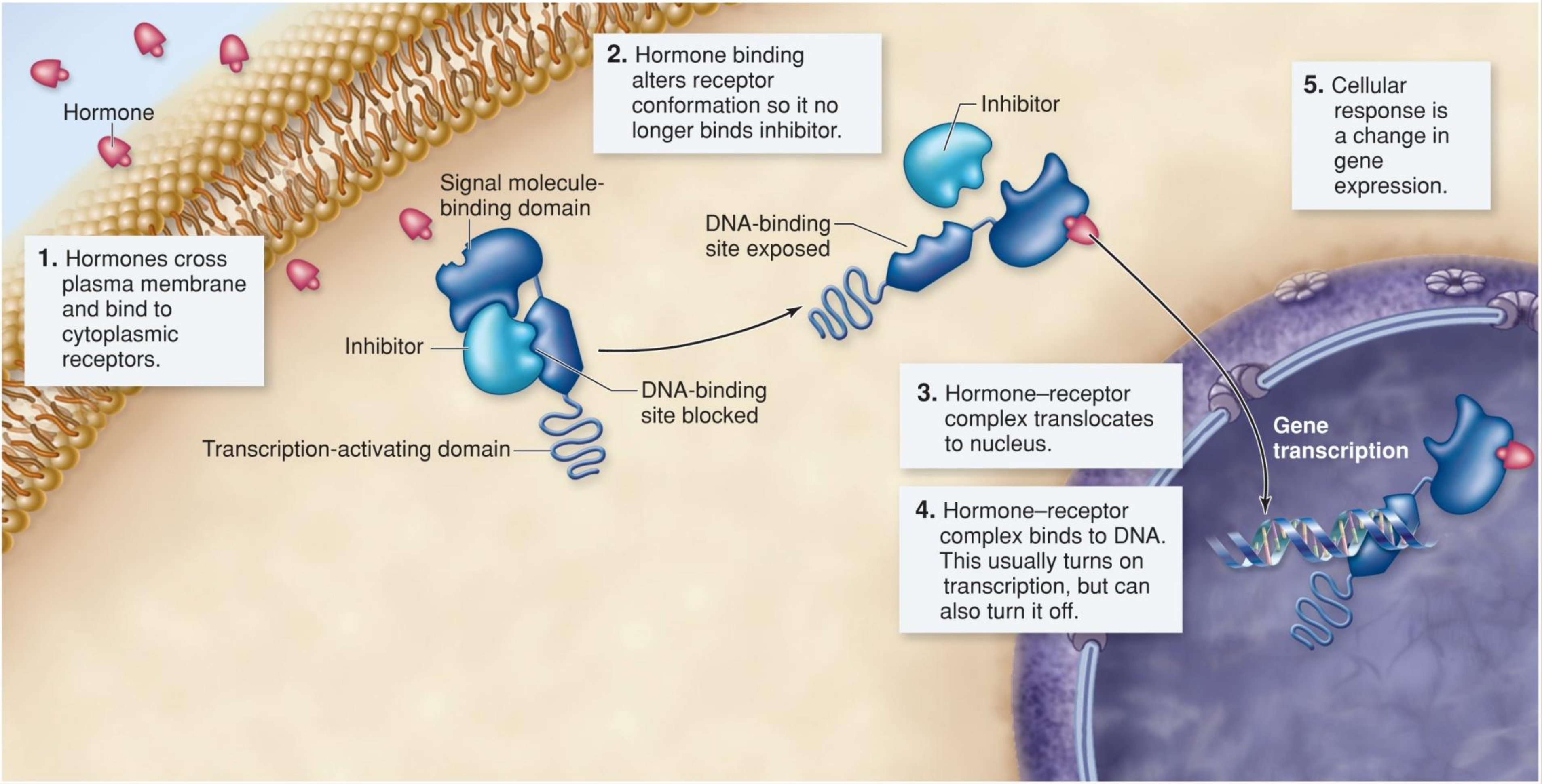


1. Hormones cross plasma membrane and bind to cytoplasmic receptors.

2. Hormone binding alters receptor conformation so it no longer binds inhibitor.

3. Hormone–receptor complex translocates to nucleus.

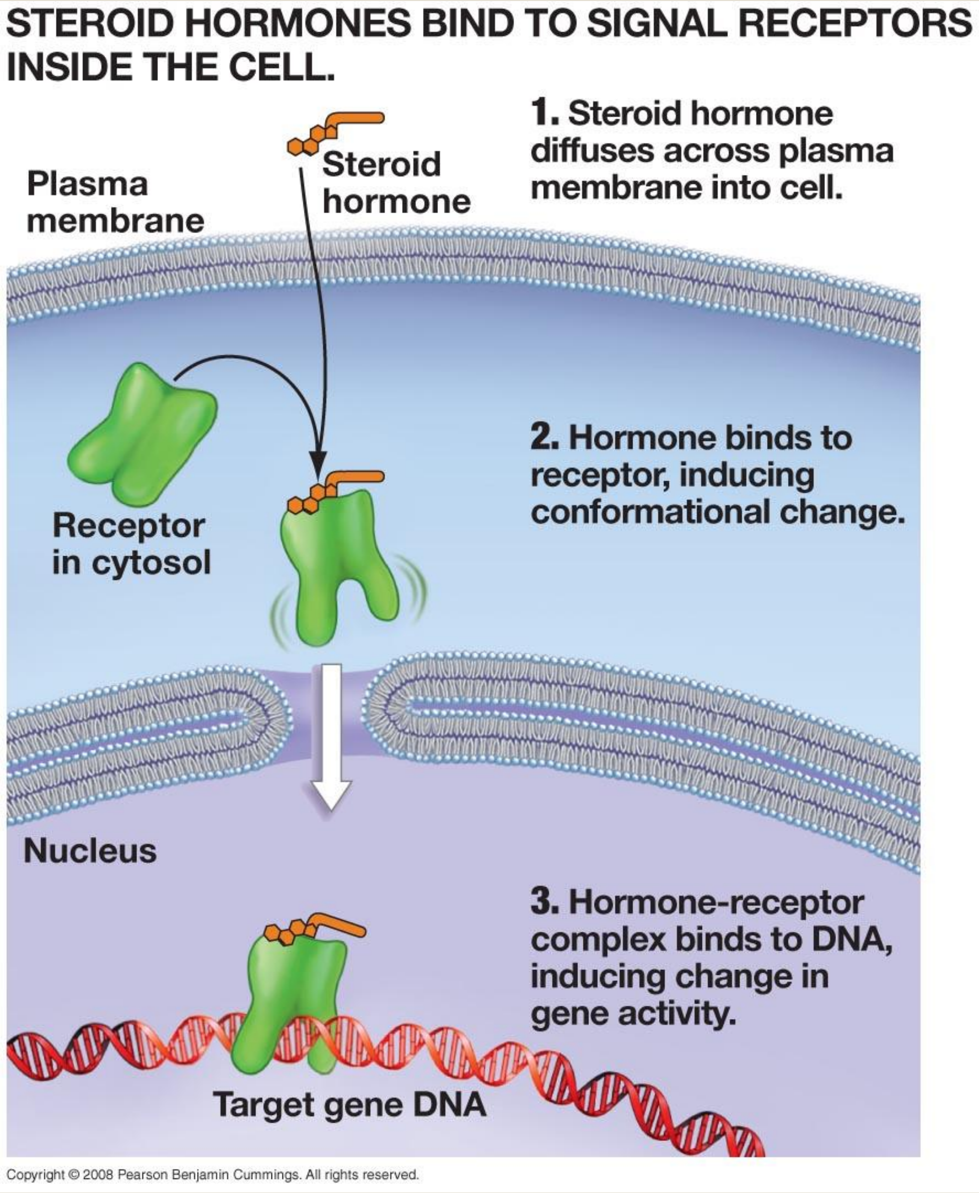
4. Hormone–receptor complex binds to DNA. This usually turns on transcription, but can also turn it off.

5. Cellular response is a change in gene expression.

Most intracellular receptors bind to DNA

Steroid Receptors

- Steroid hormones are lipids
- They can traverse the plasma membrane
- They bind to their receptor inside the cell
- Usually the receptor is a transcription factor which regulates gene expression



STEROID HORMONES BIND TO SIGNAL RECEPTORS INSIDE THE CELL.

1. Steroid hormone diffuses across plasma membrane into cell.
Steroid hormone
Plasma membrane
2. Hormone binds to receptor, inducing conformational change.
Receptor in cytosol
Nucleus
3. Hormone-receptor complex binds to DNA, inducing change in gene activity.
Target gene DNA

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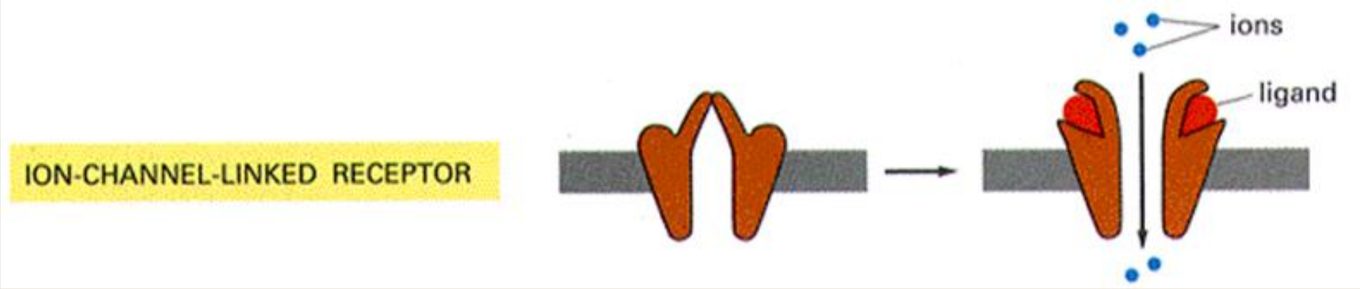
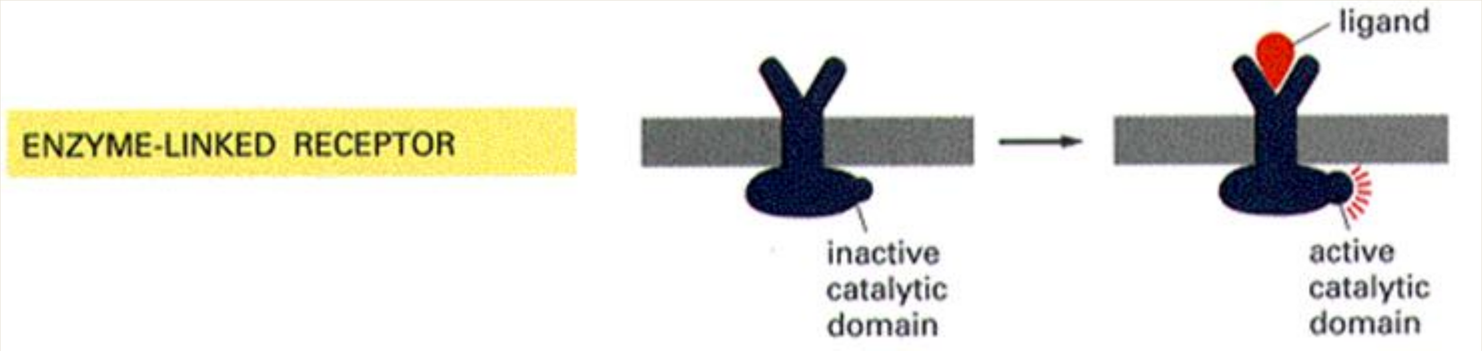
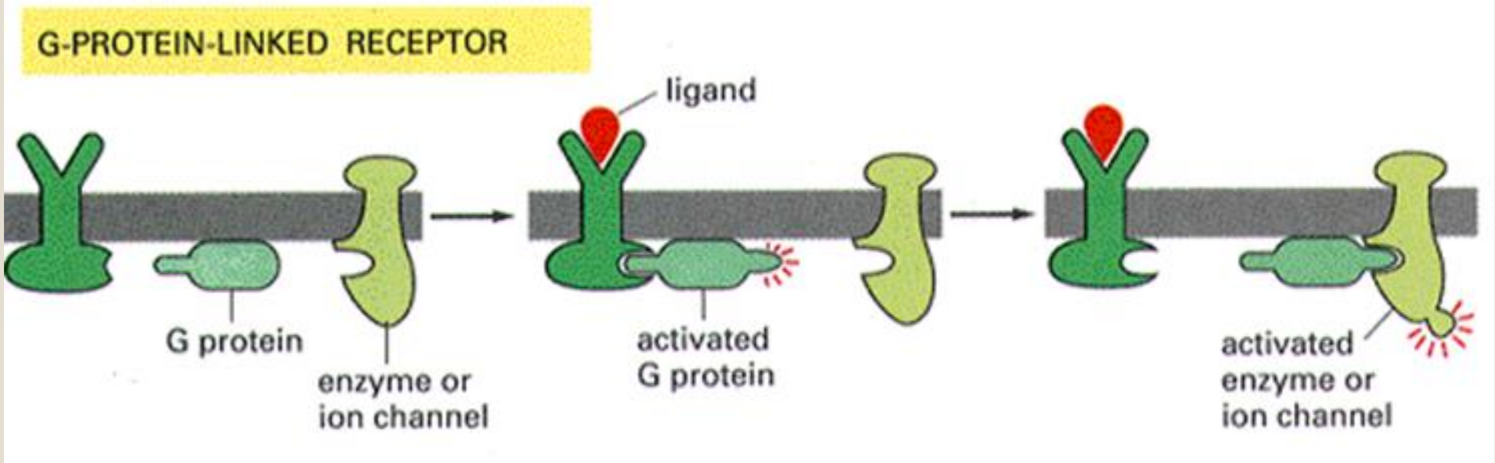
Non-Lipid Soluble Hormones

- Most hormonal signals are not lipid soluble
- Most hormonal signals need to be “transduced” to the inside of the cell
  - Known as signal transduction pathways
  - Conversion of an extracellular signal to an intracellular signal

Types of membrane receptors in signaling cascades







Models of cell surface receptors

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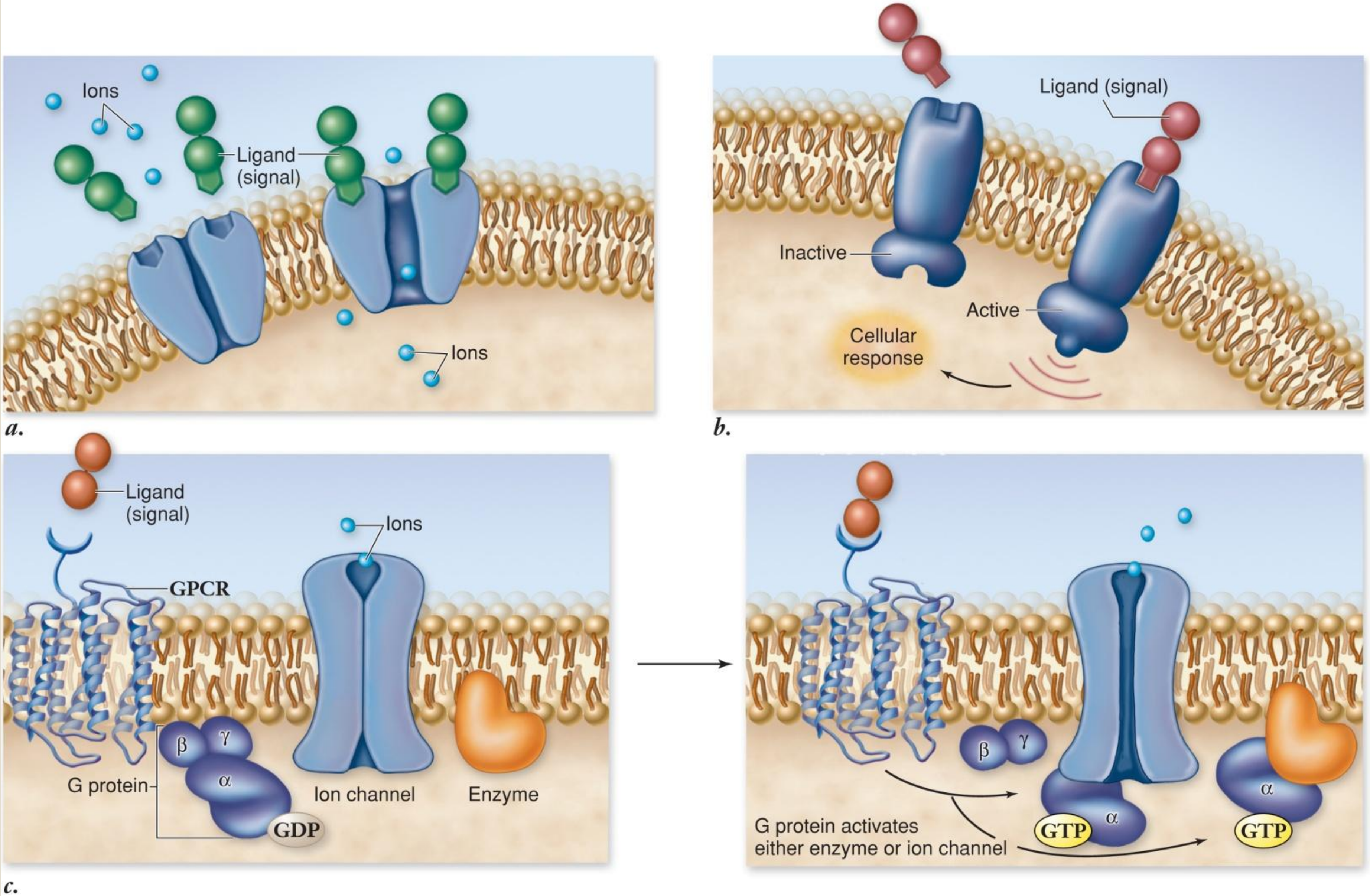

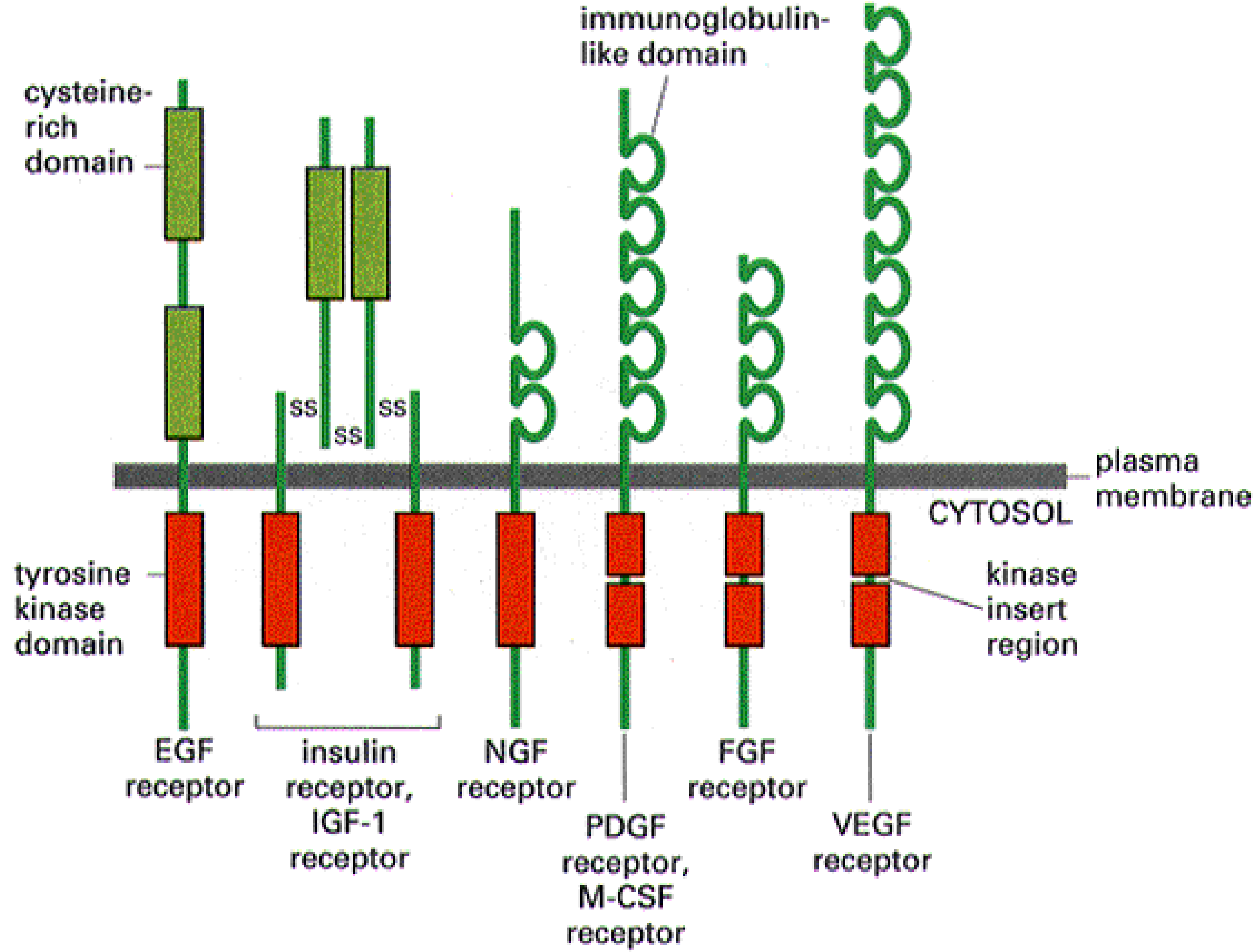

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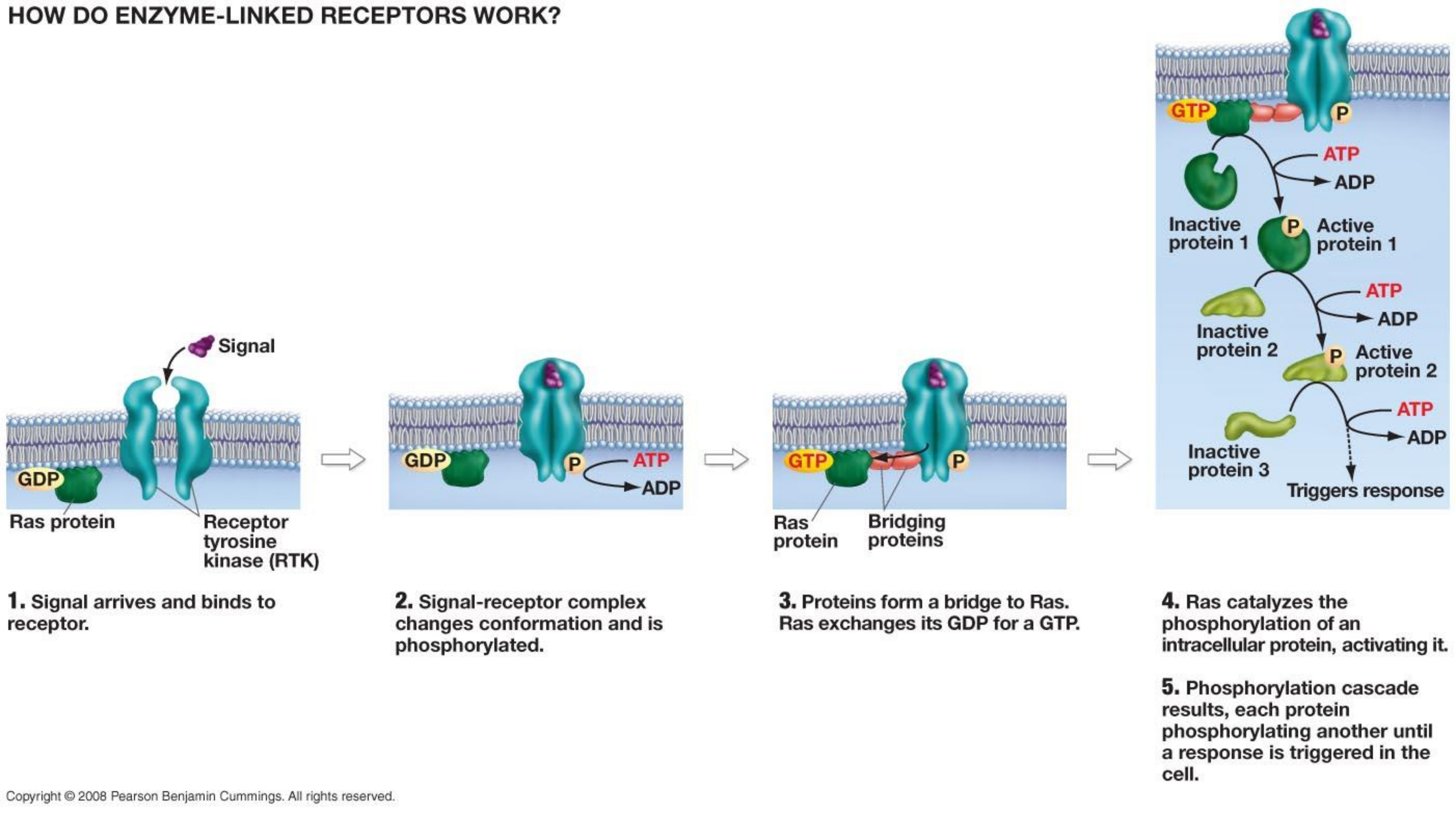
Receptor Tyrosine Kinases



HOW DO ENZYME-LINKED RECEPTORS WORK?

1. Signal arrives and binds to receptor.

2. Signal-receptor complex changes conformation and is phosphorylated.

3. Proteins form a bridge to Ras. Ras exchanges its GDP for a GTP.

4. Ras catalyzes the phosphorylation of an intracellular protein, activating it.

5. Phosphorylation cascade results, each protein phosphorylating another until a response is triggered in the cell.

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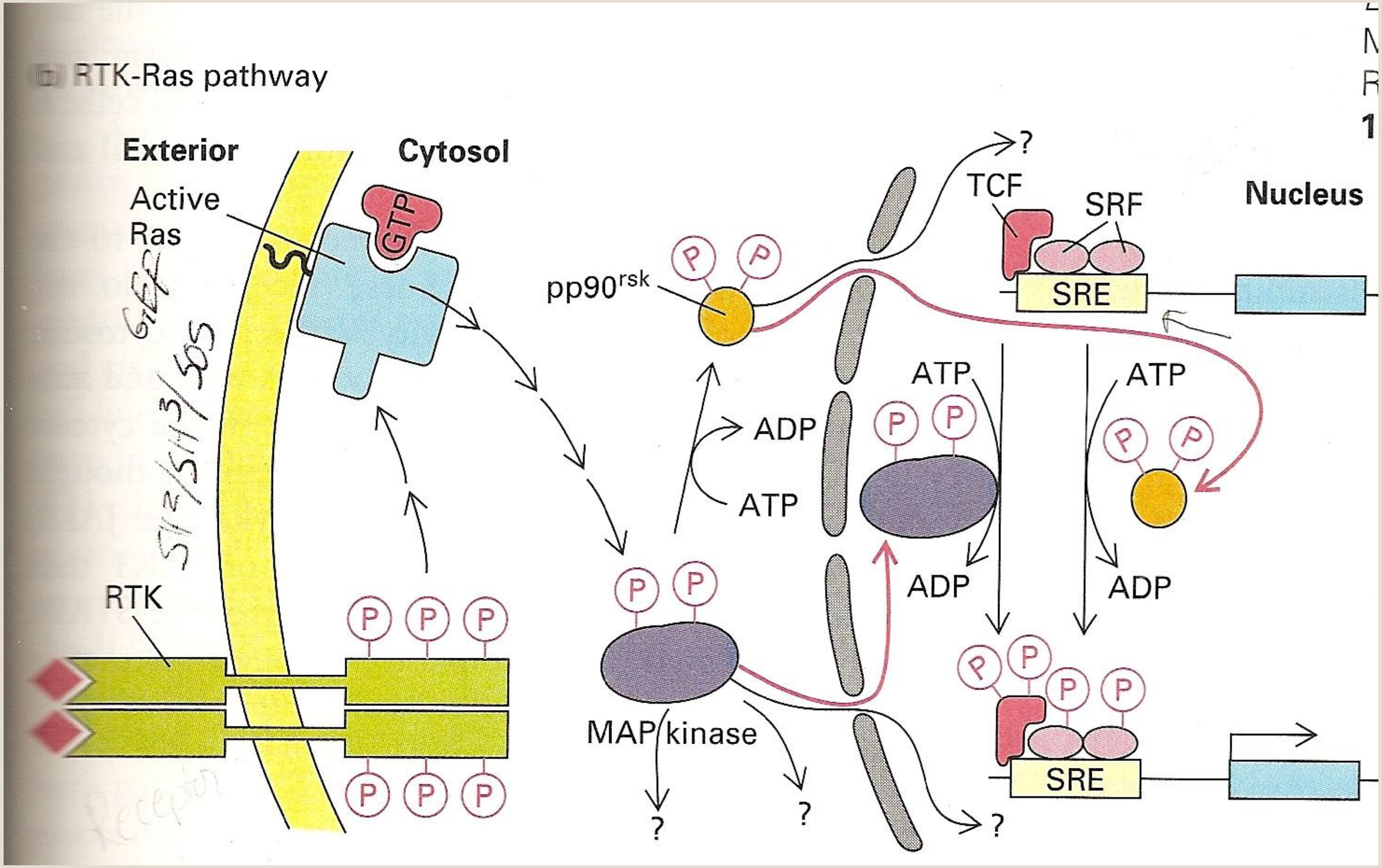
Receptor Tyrosine Kinases

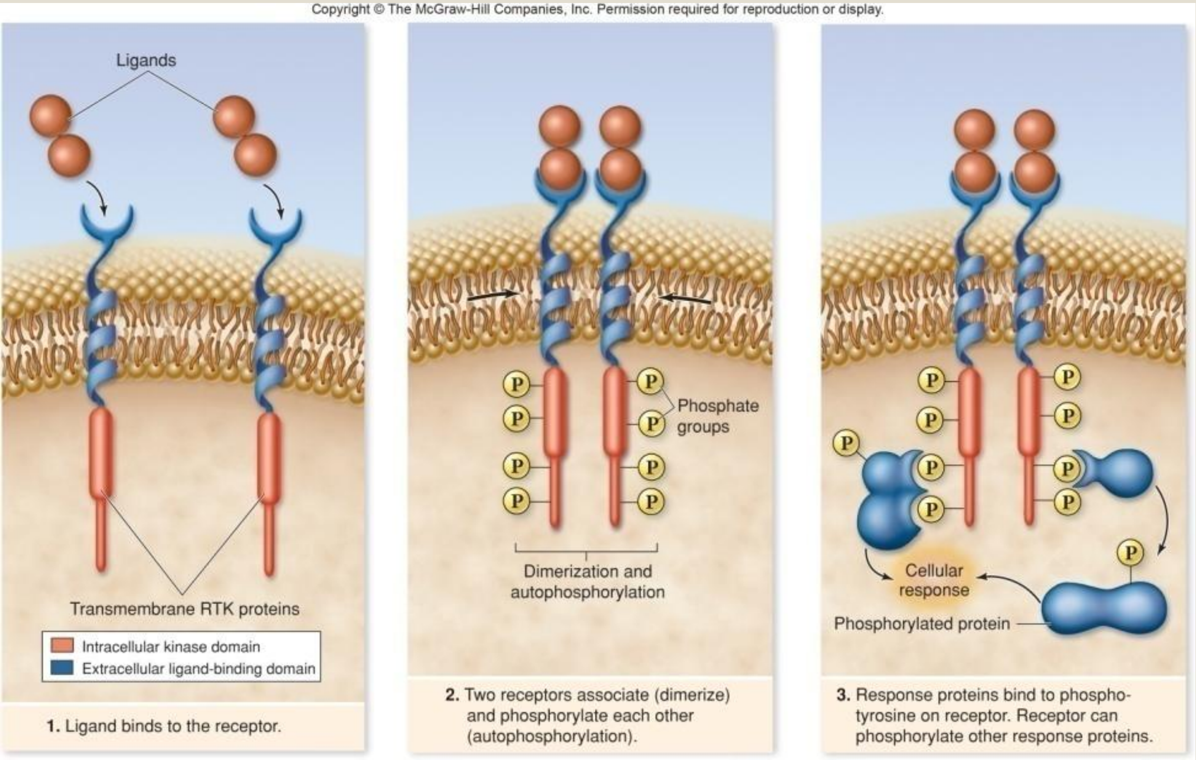
- Phosphorylation cascade
- Signal turned off by phosphatases
- Ras G-protein
  - Common point mutation makes the hydrolysis of GTP of GDP impossible
  - Therefore, GTP stays bound and ras is always activated even without hormone
  - Cancer

Receptor Tyrosine Kinases



Receptor tyrosine kinases are very common in a variety of cells

- Subunits are activated by ligand binding
- They form a dimer and phosphorylate themselves at the tyrosines
- These can trigger a variety of cellular activities-depends on the cell



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Ligands
Transmembrane RTK proteins
Intracellular kinase domain
Extracellular ligand-binding domain
P
Phosphate groups
Dimerization and autophosphorylation
Cellular response
Phosphorylated protein

1. Ligand binds to the receptor.

2. Two receptors associate (dimerize) and phosphorylate each other (autophosphorylation).

3. Response proteins bind to phosphotyrosine on receptor. Receptor can phosphorylate other response proteins.

The insulin receptor, a RTK

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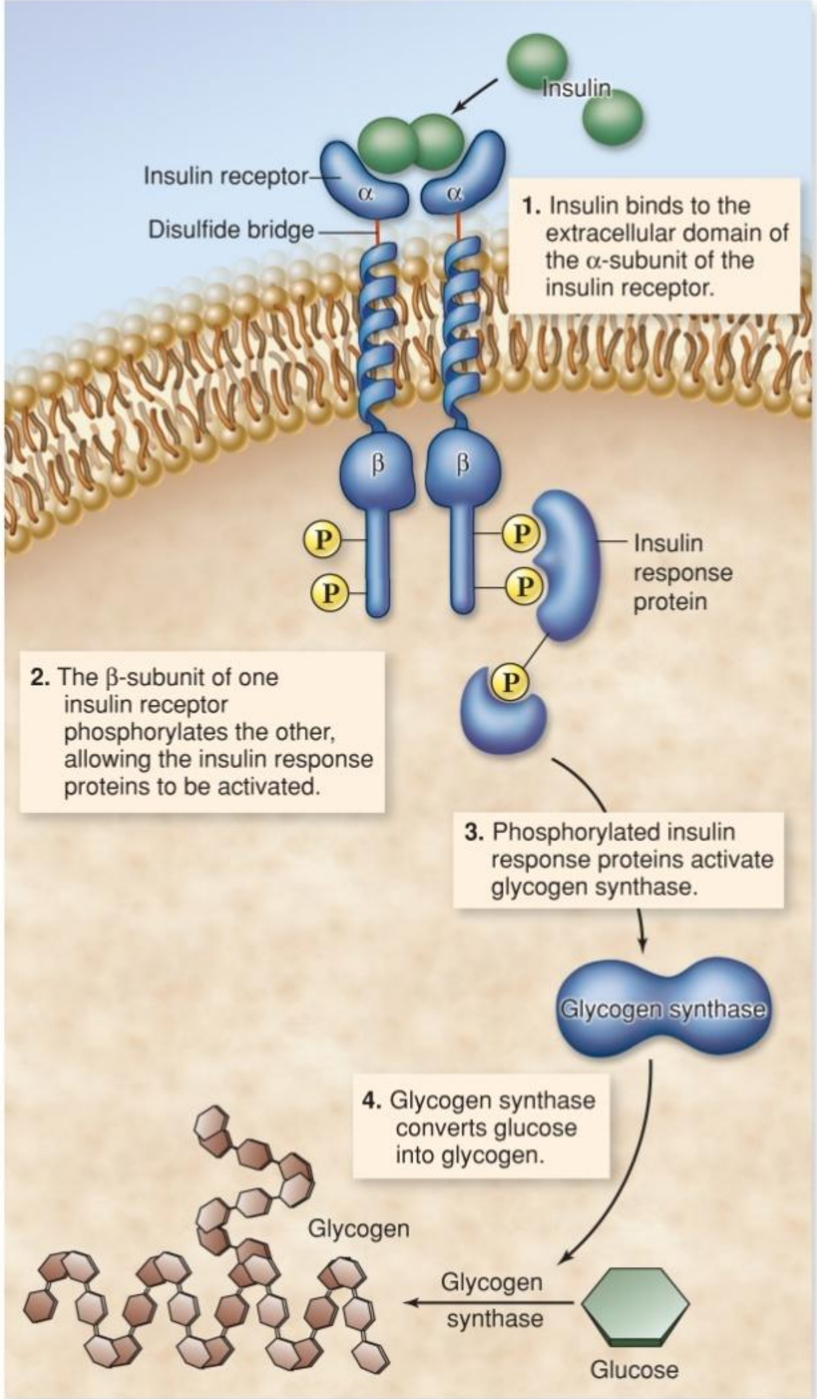

1. Insulin binds to the extracellular domain of the α-subunit of the insulin receptor.

2. The β-subunit of one insulin receptor phosphorylates the other, allowing the insulin response proteins to be activated.

3. Phosphorylated insulin response proteins activate glycogen synthase.

4. Glycogen synthase converts glucose into glycogen.

G protein-coupled receptors also regulate many cell activities

- Many are involved in taste and smell; also muscle responses
- Several hundred have been identified in humans
- G protein activation will cause an effector protein to be activated
  - Enzyme
  - Second messenger

G protein-coupled receptors

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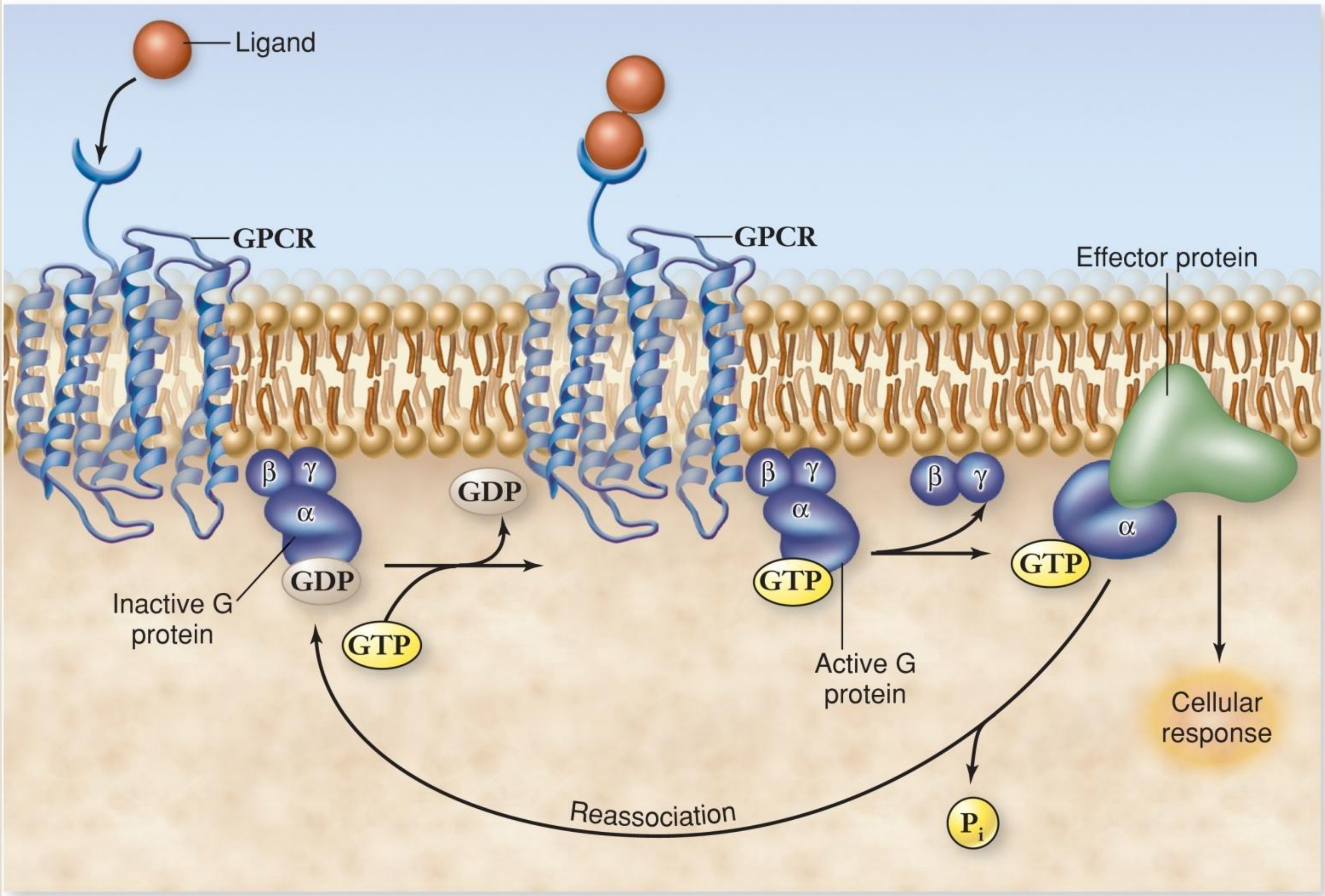
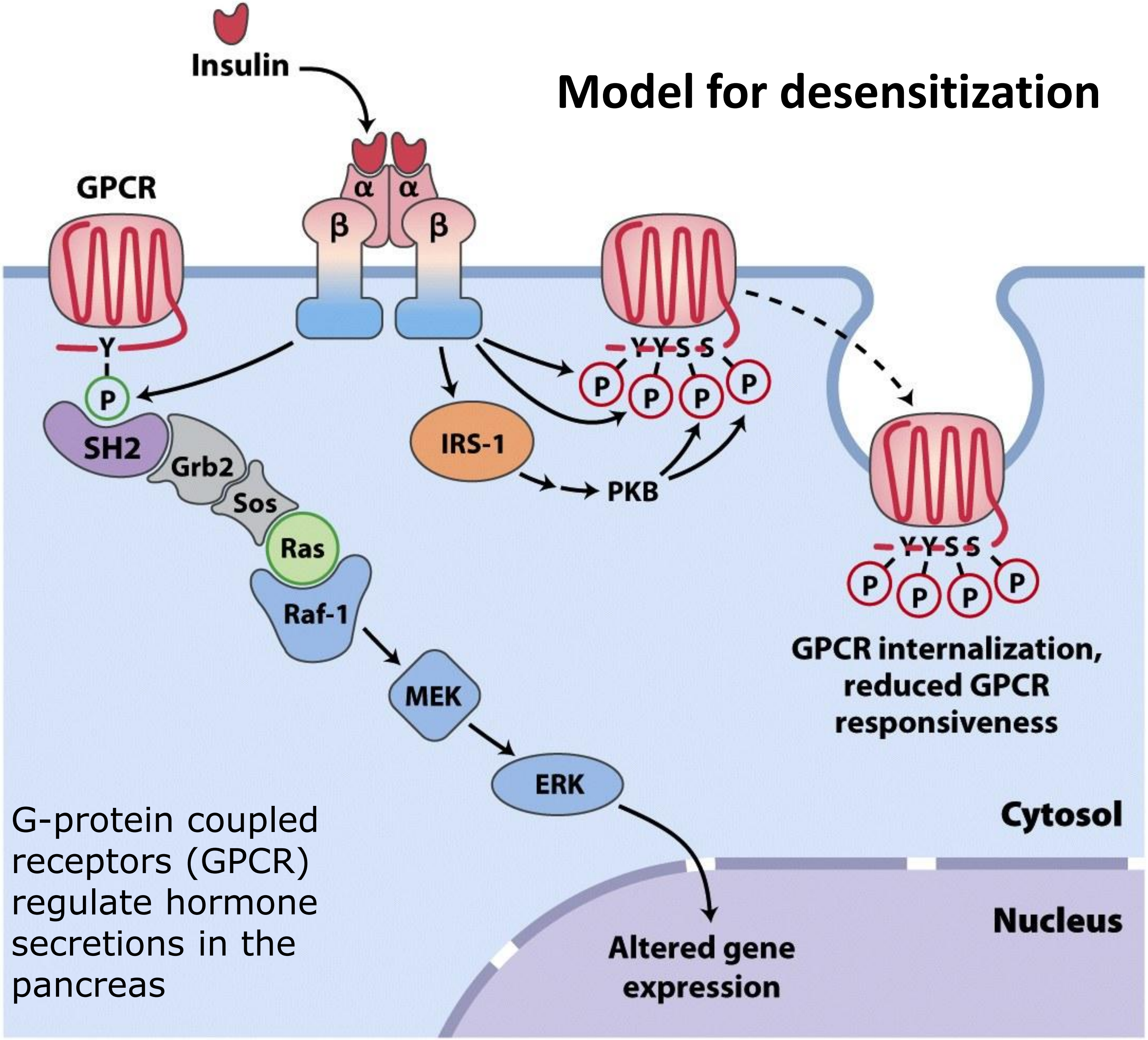


Model for desensitization



G-protein coupled receptors (GPCR) regulate hormone secretions in the pancreas

Figure 12-19
Lehninger Principles of Biochemistry, Fifth Edition
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Parameters of signal transduction

1. **Signal molecules**: properties (hydrophobicity) → location of R (surface/ intracellular)
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Assembly through specific protein binding modules/domains

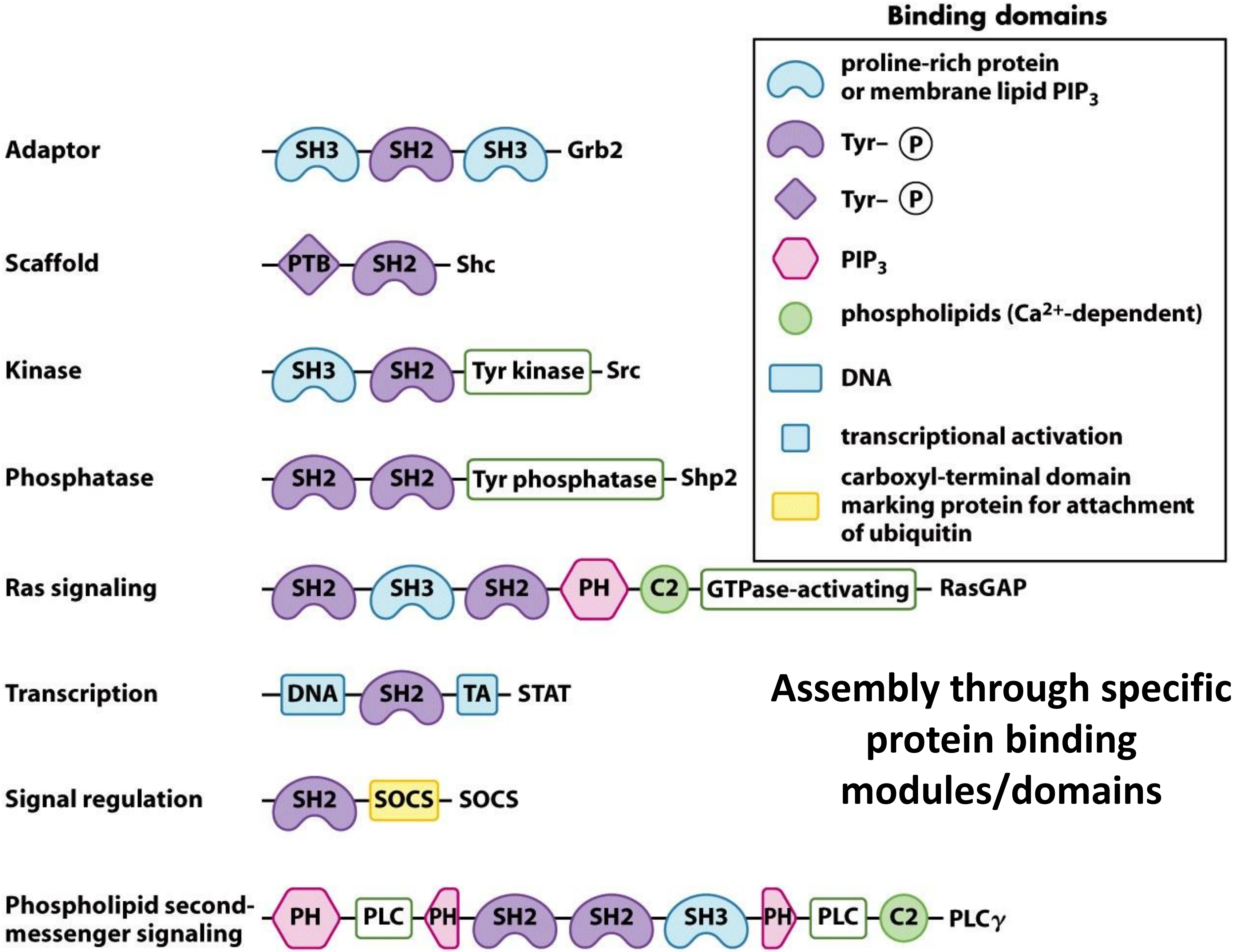
Binding domains
proline-rich protein or membrane lipid PIP_3
Tyr– (P)
Tyr– (P)
PIP_3
phospholipids (Ca^{2+}-dependent)
DNA
transcriptional activation
carboxyl-terminal domain marking protein for attachment of ubiquitin
Adaptor
SH3 SH2 SH3 Grb2
Scaffold
PTB SH2 Shc
Kinase
SH3 SH2 Tyr kinase Src
Phosphatase
SH2 SH2 Tyr phosphatase Shp2
Ras signaling
SH2 SH3 SH2 PH C2 GTPase-activating RasGAP
Transcription
DNA SH2 TA STAT
Signal regulation
SH2 SOCS SOCS
Phospholipid second-messenger signaling
PH PLC PH SH2 SH2 SH3 PH PLC C2 PLCγ

Figure 12-23
Lehninger Principles of Biochemistry, Fifth Edition
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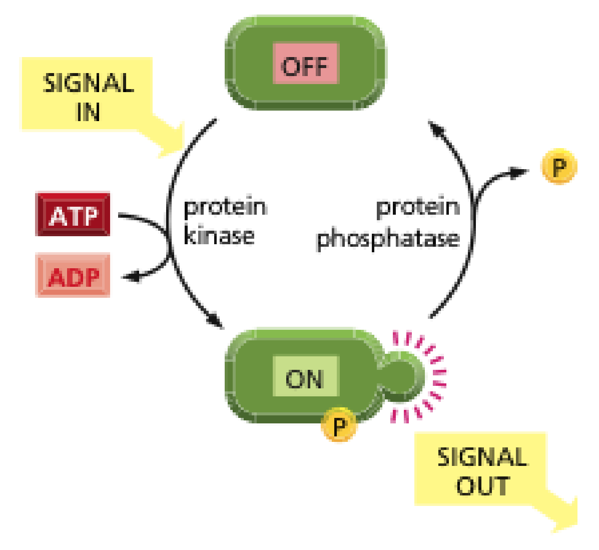
Transmitting the Signal: Protein Kinases

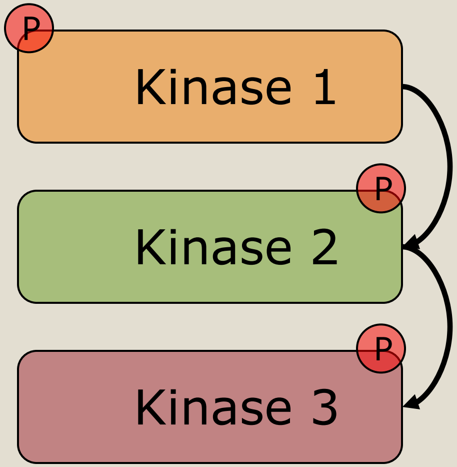
- Protein kinases are enzymes that add a phosphate group from ATP onto a substrate protein; this reaction is called phosphorylation

- Phosphorylation frequently serves to activate the substrate of the kinase, but can also target the substrate for degradation

- Kinases are often themselves activated by other kinases via phosphorylation and can organize into phosphorylation cascades

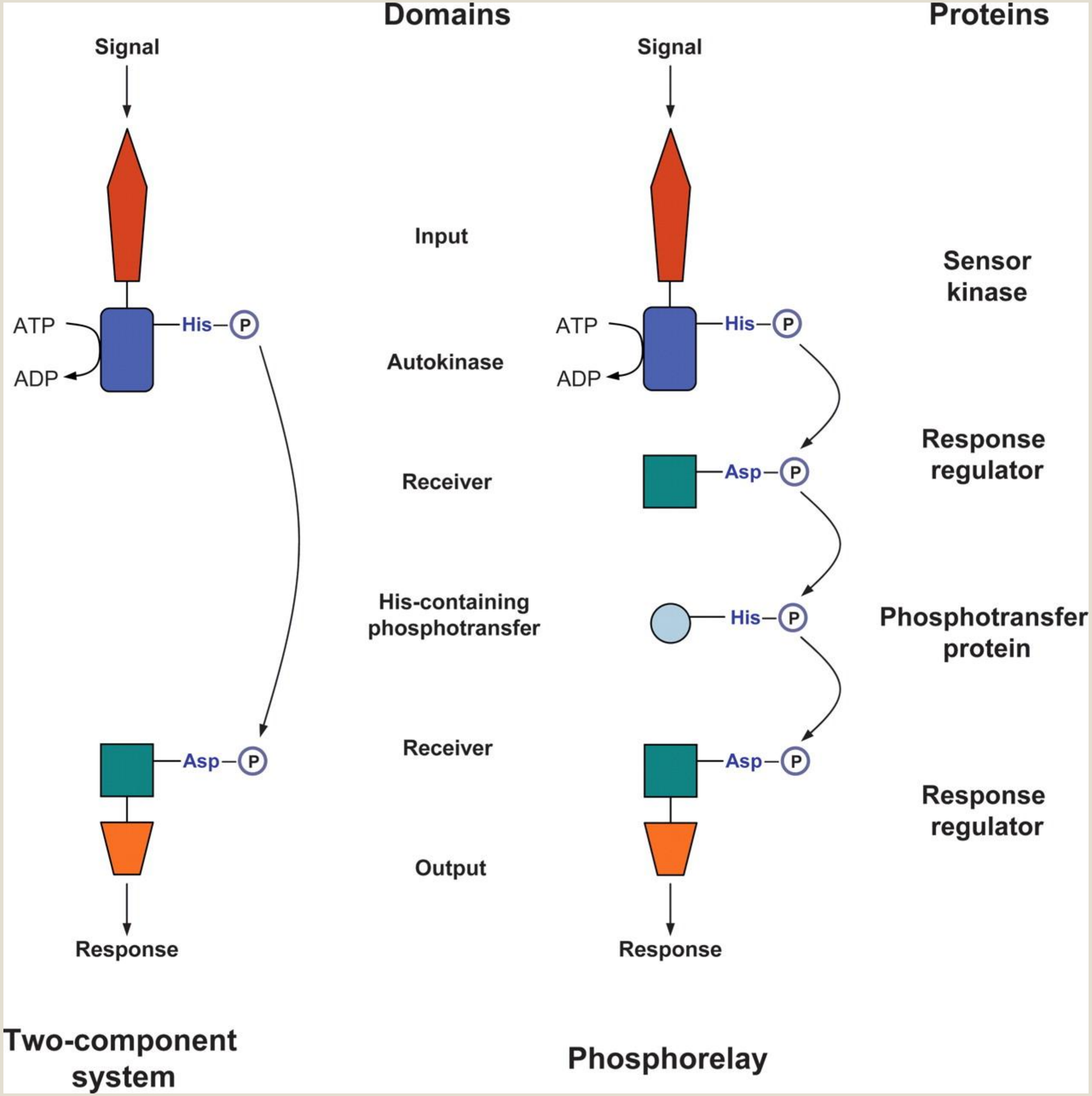
- One important class of phosphorylation cascade is called a mitogen activated protein kinase (MAPK) cascade




P
Kinase 1
P
Kinase 2
P
Kinase 3

Phosphorylation Cascade

Schematics of the proteins and domains that constitute TCSs (left) and phosphorelays (right).



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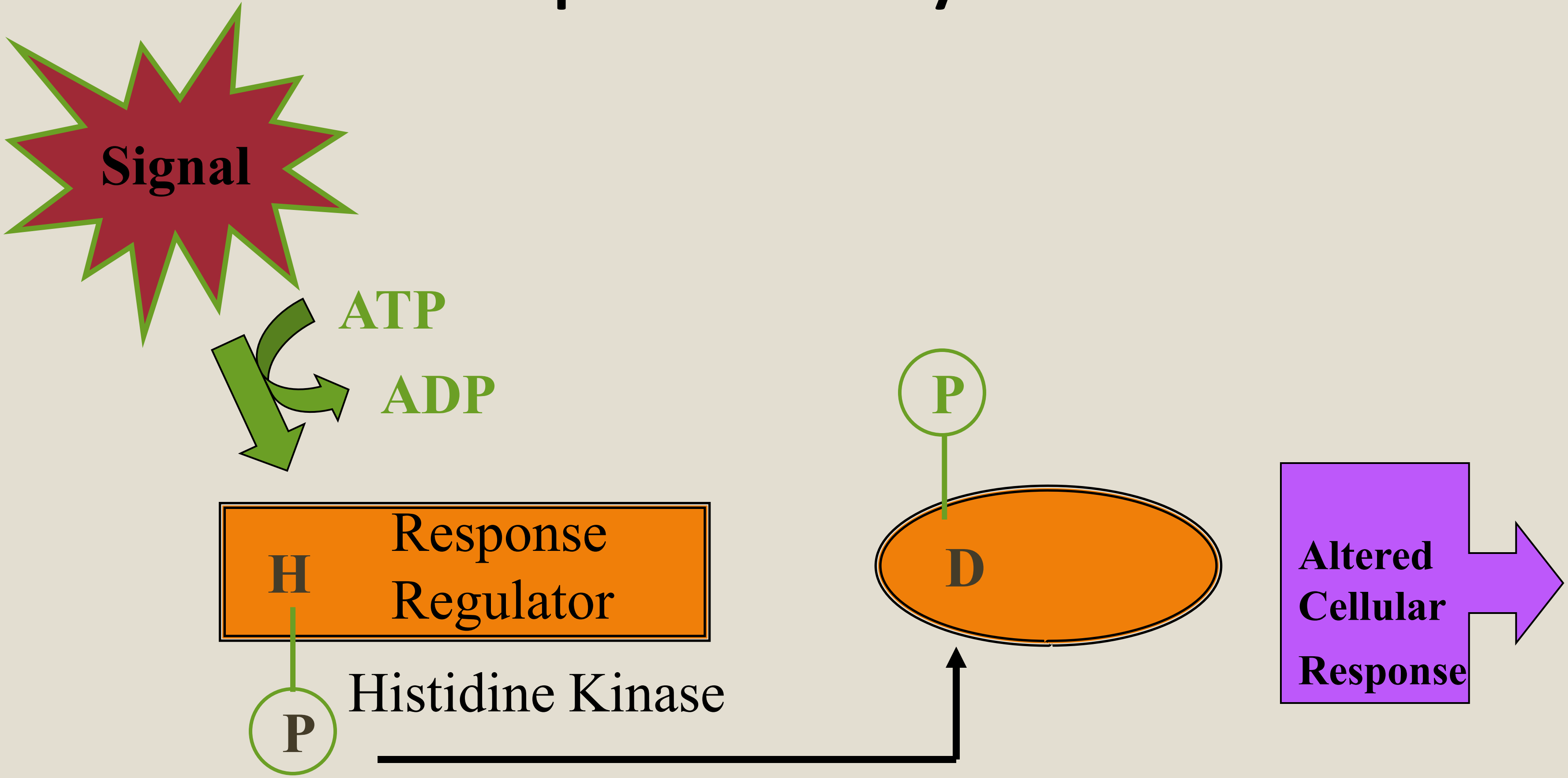
Mitrophanov A Y , Groisman E A Genes Dev. 2008;22:2601-2611

Signal transduction systems in prokaryotes

- In prokaryotes, signal transduction typically involves **two-component regulatory systems**, which include
- a membrane-integrated **sensor kinase protein** and a cytoplasmic **response regulator protein.**
- The activity of the response regulator depends on its state of phosphorylation**.**

2-Component Systems



Signal

ATP

ADP

H

Response

Regulator

P

Histidine Kinase

P

D

Altered

Cellular

Response

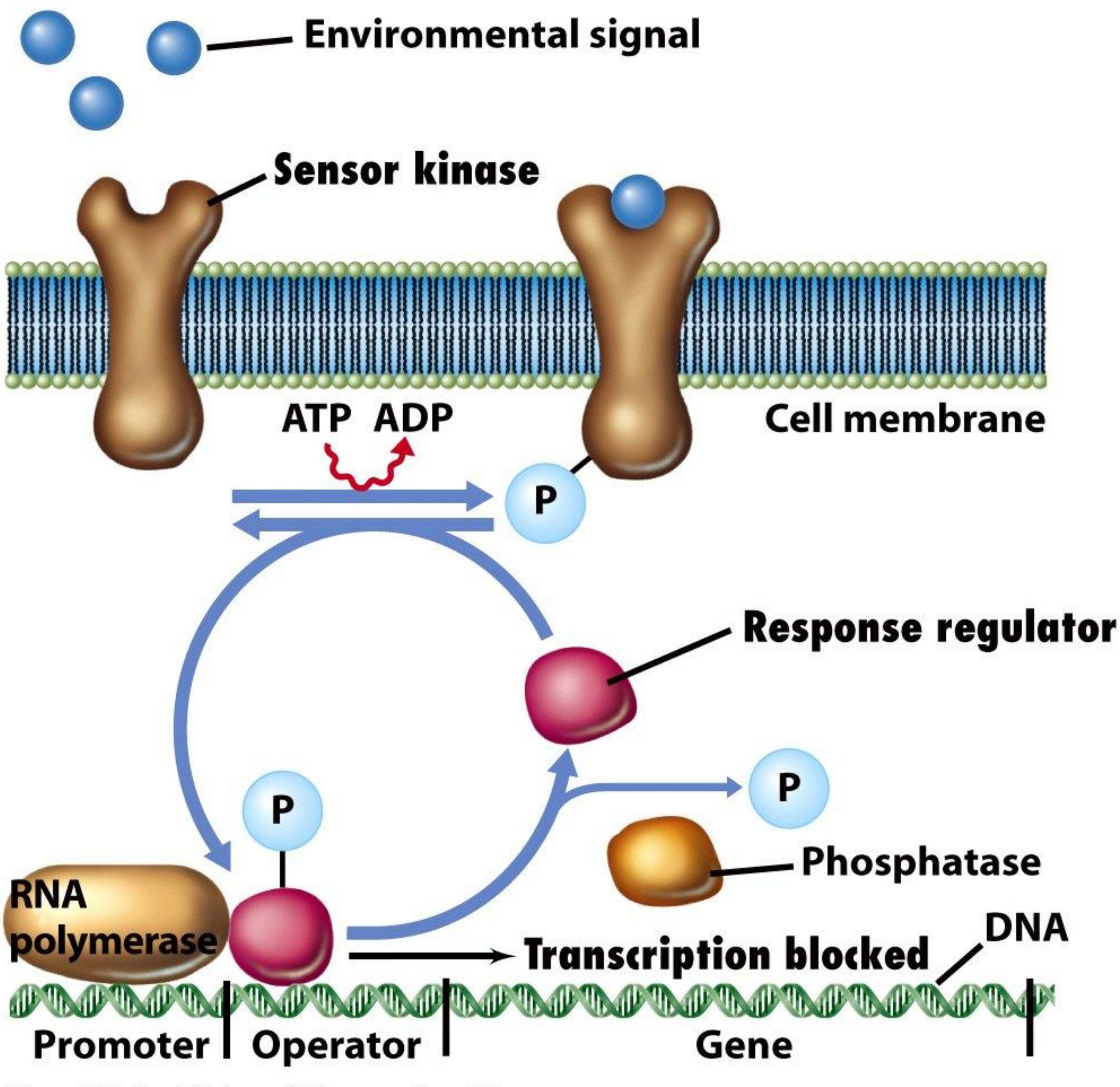


Figure 8-26 Brock Biology of Microorganisms 11/e

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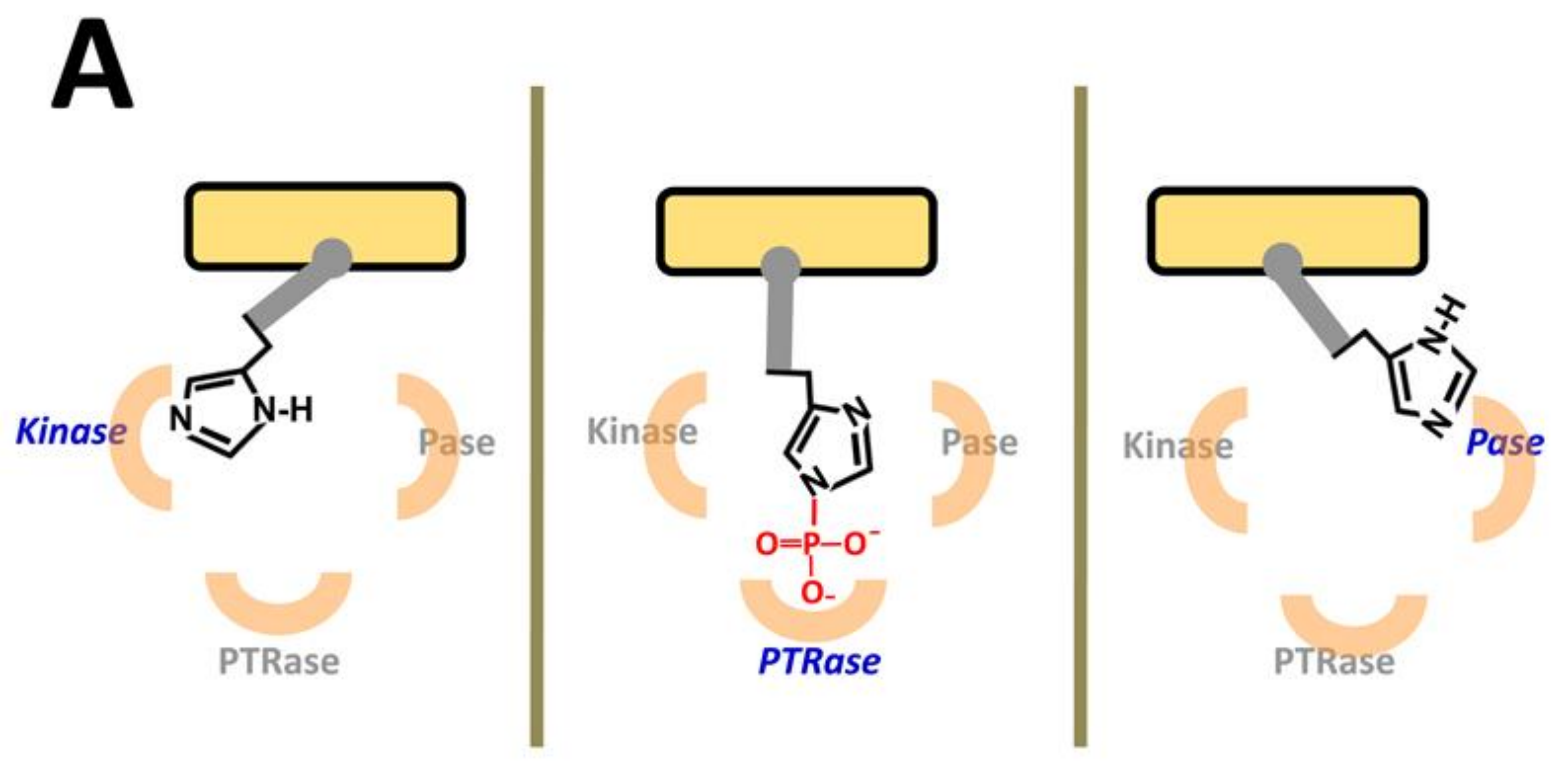
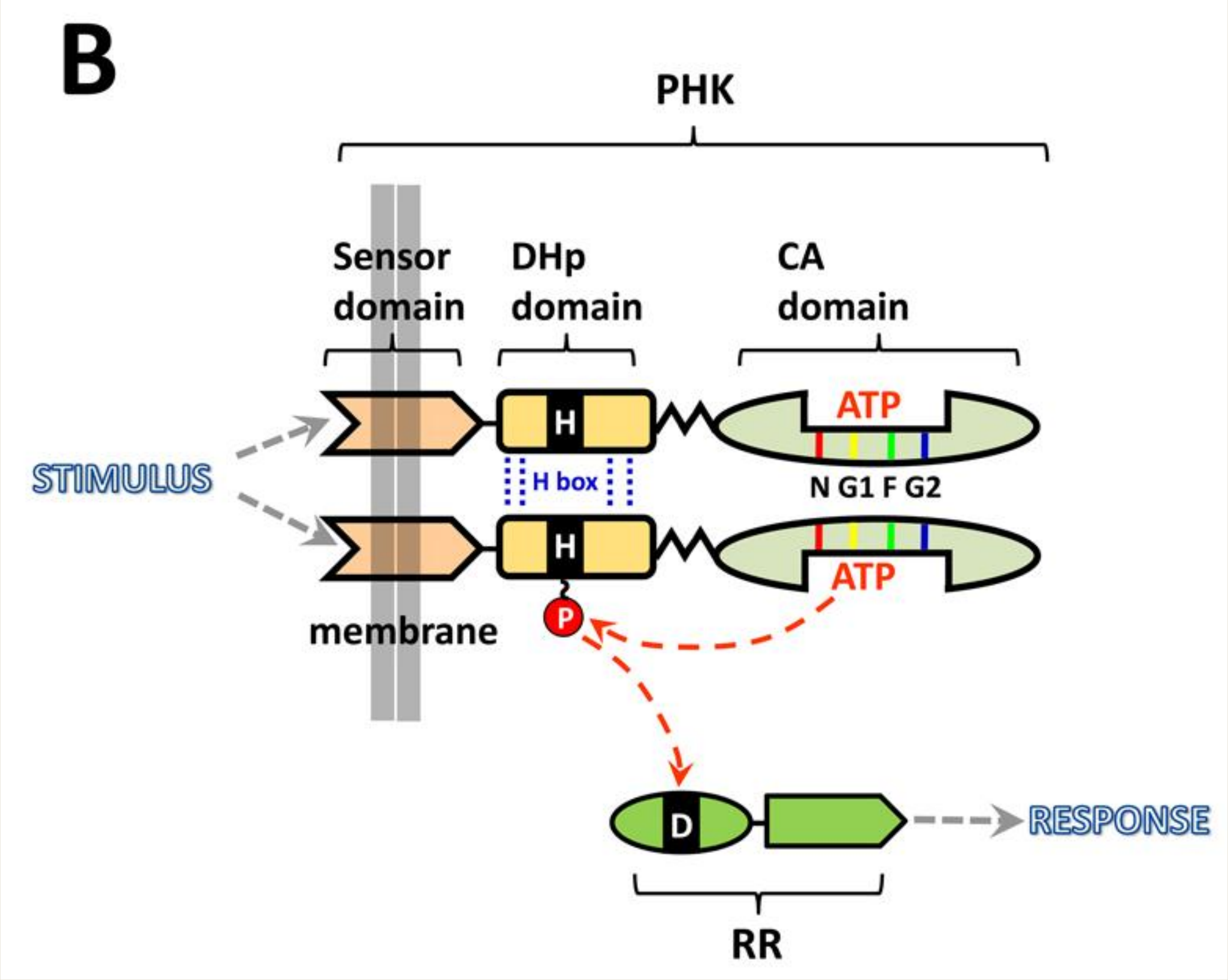
Schematic diagram of the role played by PHKs in two-component signal transduction systems (TCSs).



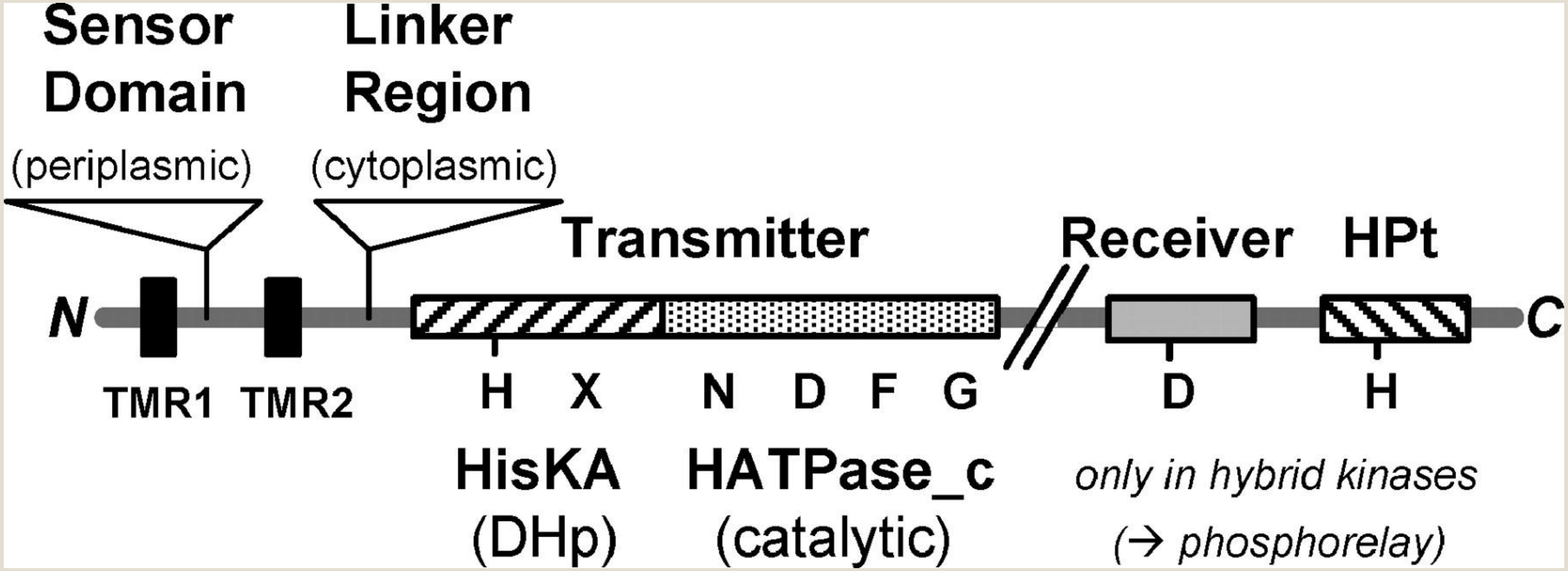
Curr Opin Microbiol. 2010 April; 13(2): 133–141.

(A) Many PHKs have three distinct but interrelated enzymatic activities that involve positioning the phospho-accepting His in three active sites. The active sites for phosphotransferase (PTRase) and phosphatase(Pase) activities are likely to be very similar but are portrayed as being physically distinct for the purpose of illustration.

(B) PHKs function as homodimers that autophosphorylate then pass their phosphoryl group to an aspartate side chain located in the receiver domain of a cognate response regulator protein. Each PHK monomer has three distinct structural/functional domains: a transmembrane sensor, a DHp domain, and a CA domain.



Features, domains, and boxes of histidine protein kinases.



Mascher T et al. Microbiol. Mol. Biol. Rev. 2006;70:910-938

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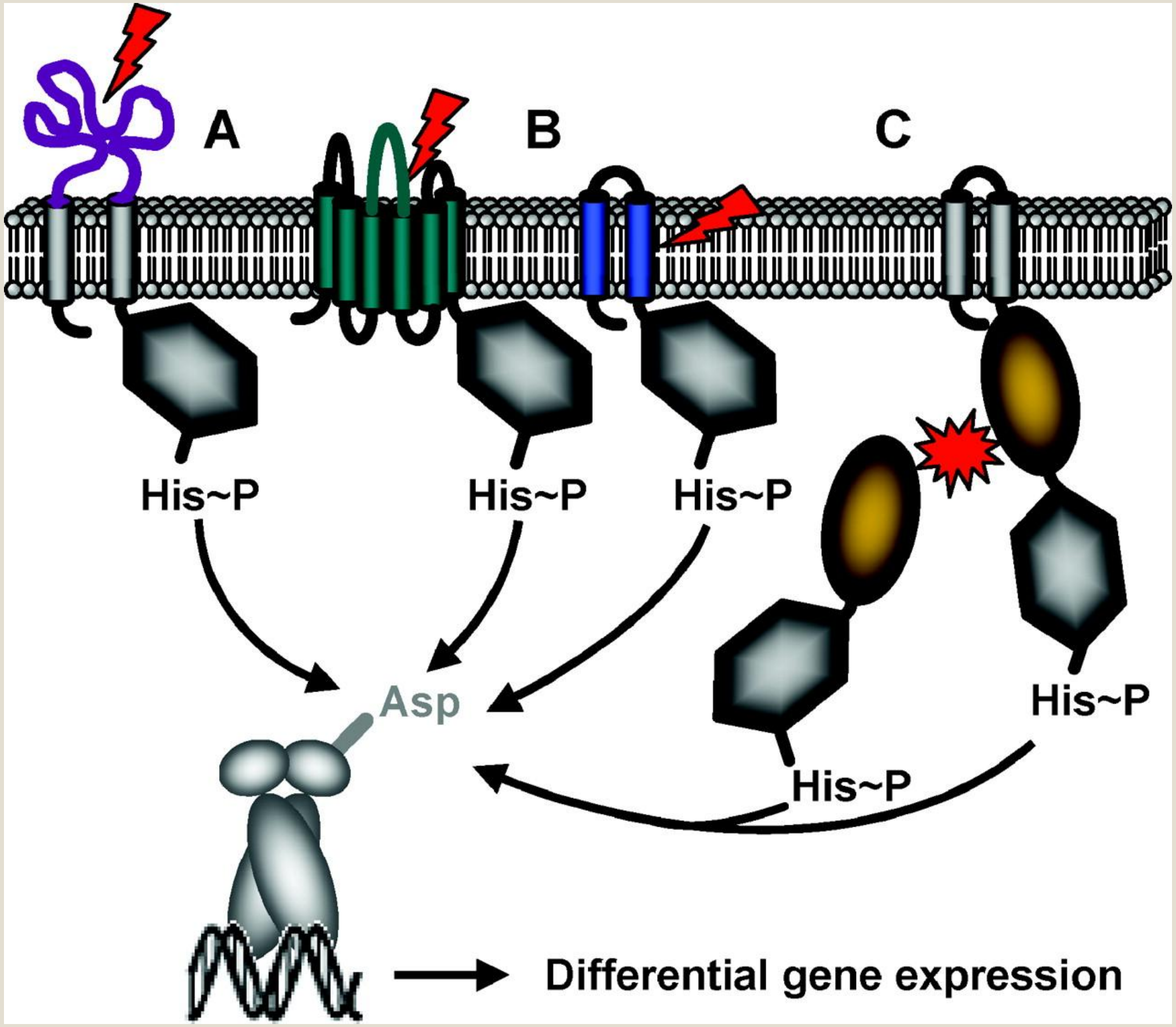
Schematic representation of the three different mechanisms of stimulus perception.



Mascher T et al. Microbiol. Mol. Biol. Rev. 2006;70:910-938

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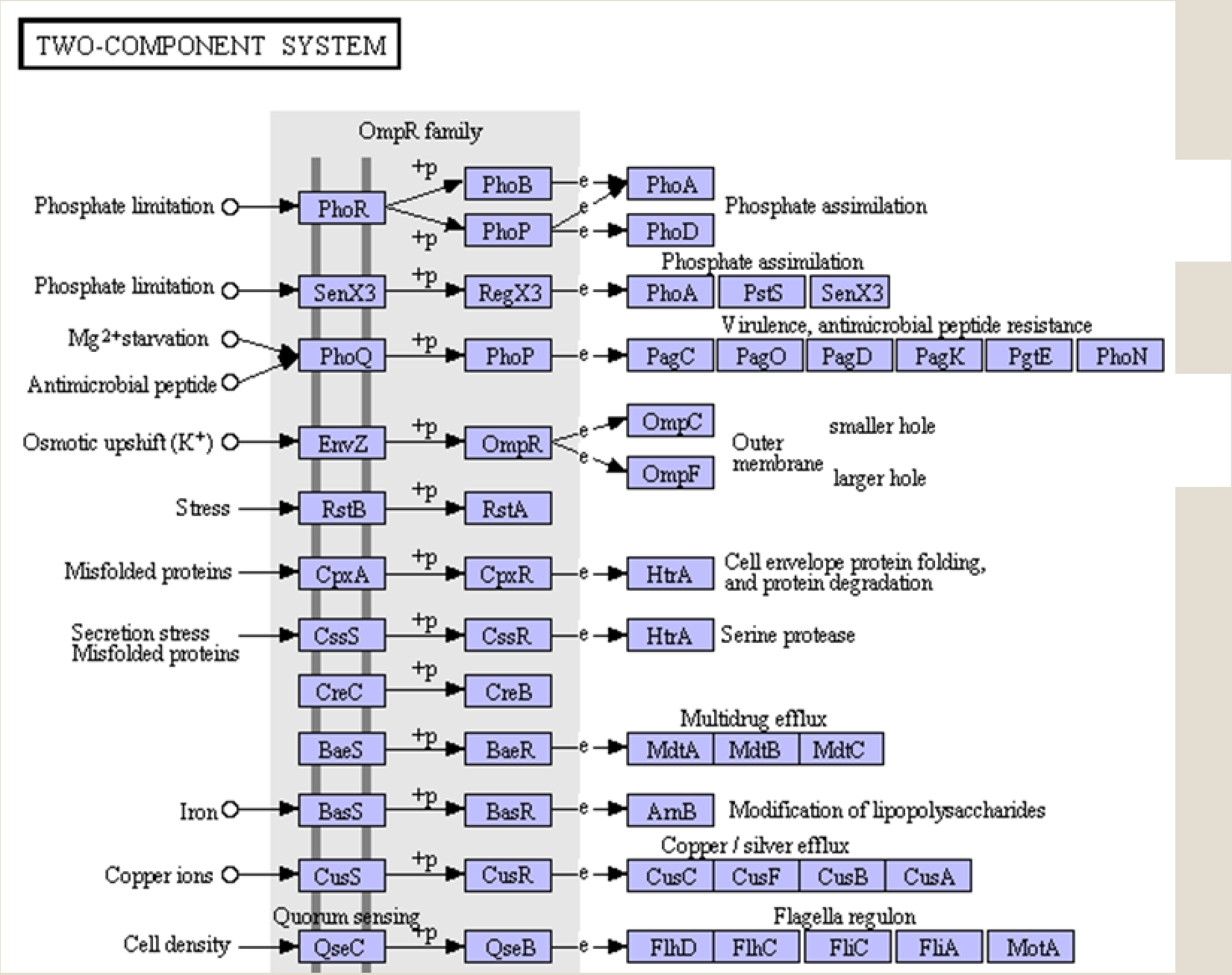
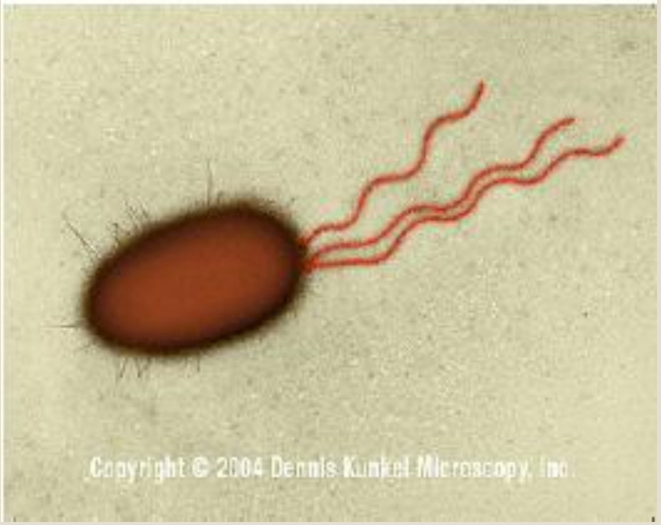

TWO-COMPONENT SYSTEM
OmpR family
Phosphate limitation
PhoR
+p
PhoB
PhoP
e
PhoA
PhoD
Phosphate assimilation
Phosphate limitation
SenX3
RegX3
Phosphate assimilation
PhoA
PstS
SenX3
Mg 2+starvation
Antimicrobial peptide
PhoQ
PhoP
Virulence, antimicrobial peptide resistance
PagC
PagO
PagD
PagK
PgtE
PhoN
Osmotic upshift (K+)
EnvZ
OmpR
OmpC
OmpF
Outer membrane
smaller hole
larger hole
Stress
RstB
RstA
Misfolded proteins
CpxA
CpxR
HtrA
Cell envelope protein folding, and protein degradation
Secretion stress
Misfolded proteins
CssS
CssR
HtrA
Serine protease
CreC
CreB
Multidrug efflux
BaeS
BaeR
MdtA
MdtB
MdtC
Iron
BasS
BasR
ArnB
Modification of lipopolysaccharides
Copper / silver efflux
Copper ions
CusS
CusR
CusC
CusF
CusB
CusA
Quorum sensing
Cell density
QseC
QseB
Flagella regulon
FlhD
FlhC
FliC
FliA
MotA

Responses Mediated by 2-Component Systems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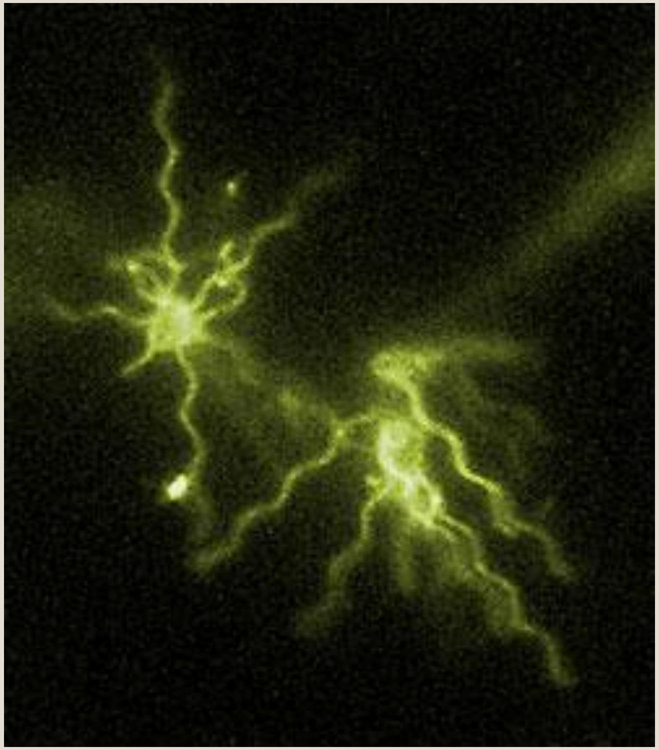
- Bacteria
  - Adaptation to changing osmolarity
  - Sporulation
  - Chemotaxis
  - N_2 metabolism
  - Redox status of cell
  - Light
  - Virulence and more



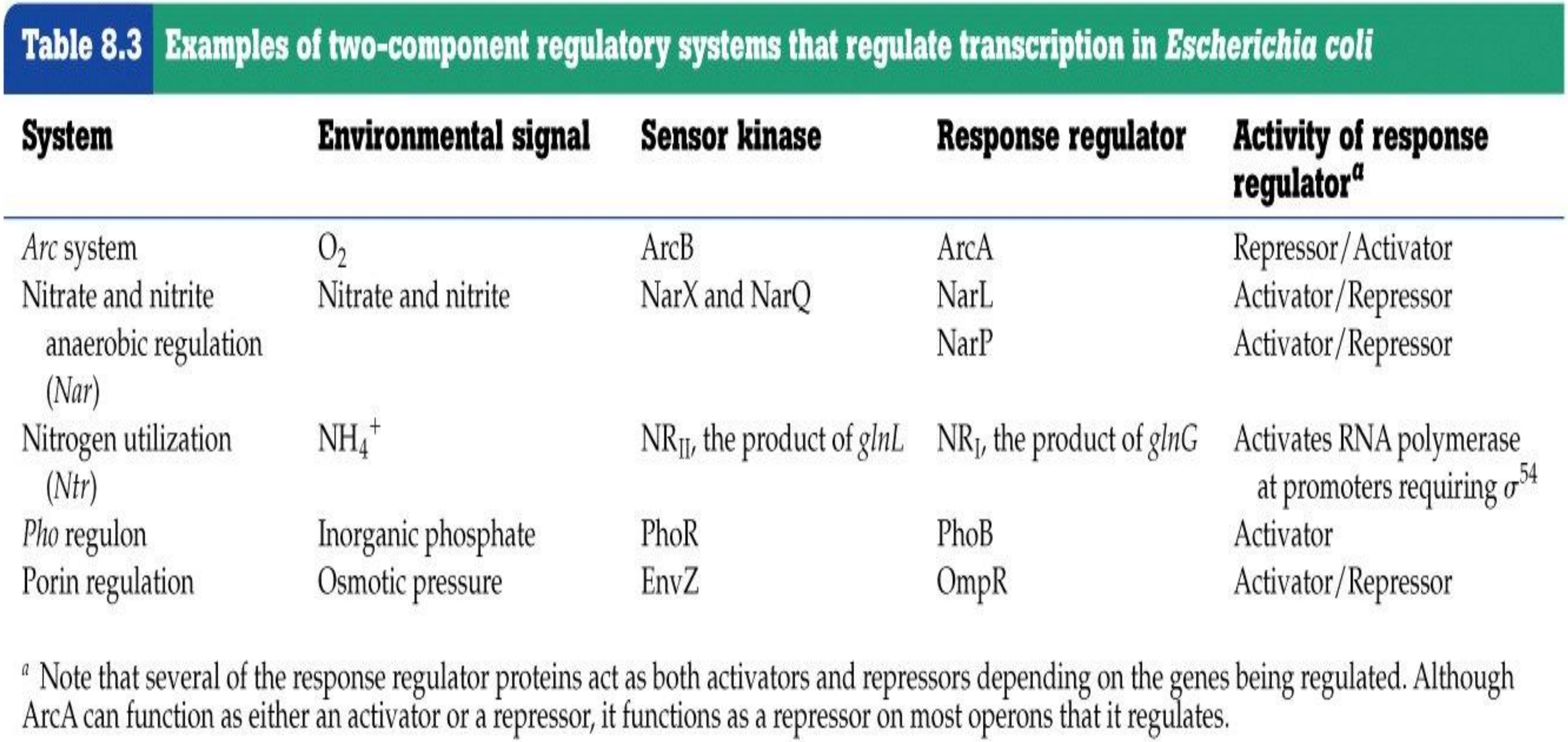


Table 8.3 Examples of two-component regulatory systems that regulate transcription in *Escherichia coli*

System	Environmental signal	Sensor kinase	Response regulator	Activity of response regulator[a]
Arc system	O_2	ArcB	ArcA	Repressor/Activator
Nitrate and nitrite anaerobic regulation (*Nar*)	Nitrate and nitrite	NarX and NarQ	NarL	Activator/Repressor
			NarP	Activator/Repressor
Nitrogen utilization (*Ntr*)	NH_4^+	NR_{II}, the product of *glnL*	NR_I, the product of *glnG*	Activates RNA polymerase at promoters requiring σ^{54}
Pho regulon	Inorganic phosphate	PhoR	PhoB	Activator
Porin regulation	Osmotic pressure	EnvZ	OmpR	Activator/Repressor

[a] Note that several of the response regulator proteins act as both activators and repressors depending on the genes being regulated. Although ArcA can function as either an activator or a repressor, it functions as a repressor on most operons that it regulates.

Table 8-3 Brock Biology of Microorganisms 11/e

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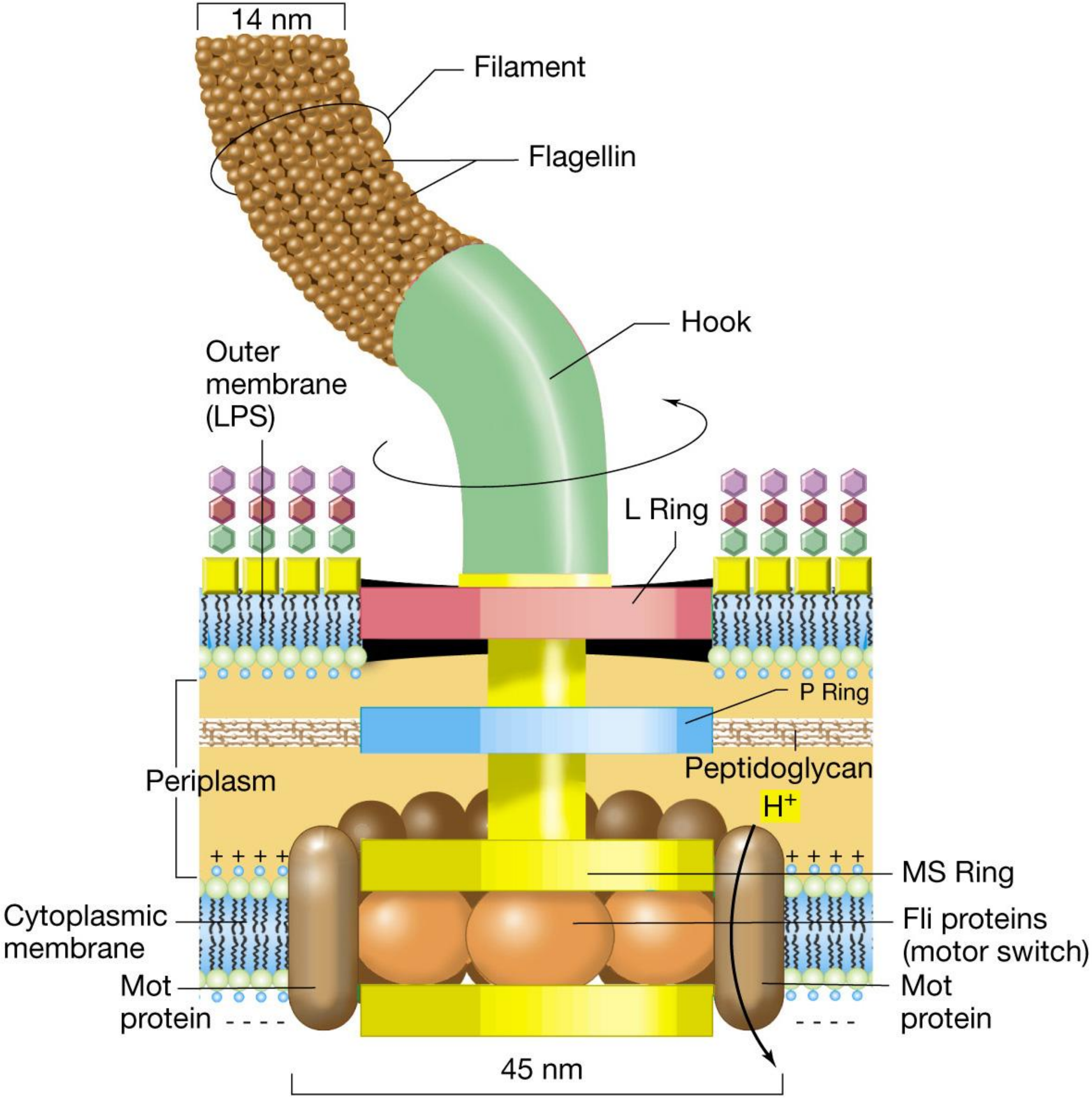
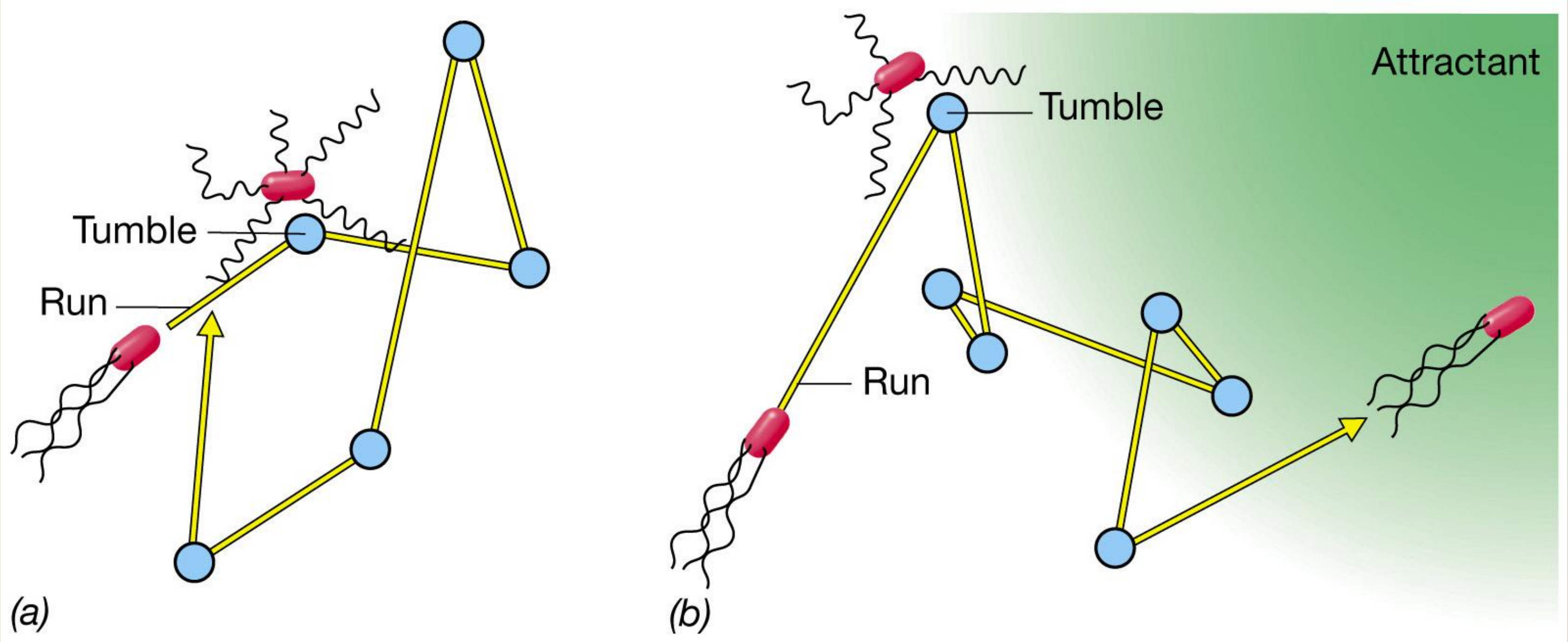
Chemotaxis and other taxes

- Movement in response to environmental stimulus
  - Positive chemotaxis, attraction towards nutrients
  - Negative: away from harmful chemicals
  - Aerotaxis: motility in response to oxygen
  - Phototaxis: motility to certain wavelengths of light
  - Magnetotaxis: response to magnetic fields

- Taxis is movement
  - Includes swimming through liquid using flagella
  - Swarming over surfaces with flagella
  - Gliding motility, requiring a surface to move over

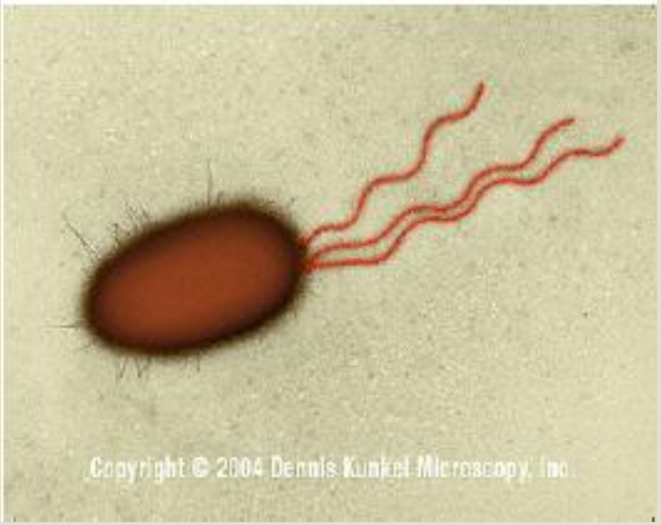

14 nm
Filament
Flagellin
Hook
Outer membrane (LPS)
L Ring
P Ring
Peptidoglycan
Periplasm
H+
MS Ring
Cytoplasmic membrane
Fli proteins (motor switch)
Mot protein
Mot protein
45 nm

Chemotaxis in a peritrichously flagellated bacterium (*E. coli)*



(a) In the absence of a chemical attractant the cell swims randomly in runs, changing direction during tumbles. (b) In the presence of an attractant runs become biased, and the cell moves up the gradient of the attractant.

Robustness in bacterial chemotaxis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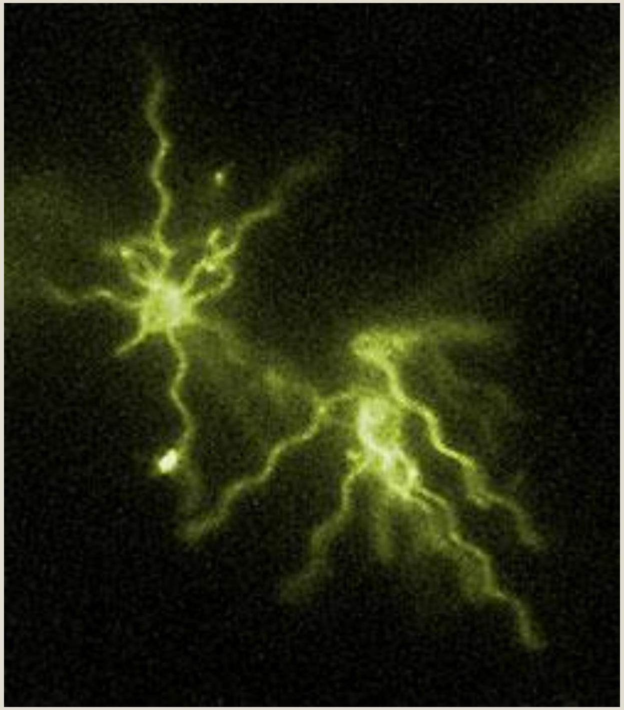
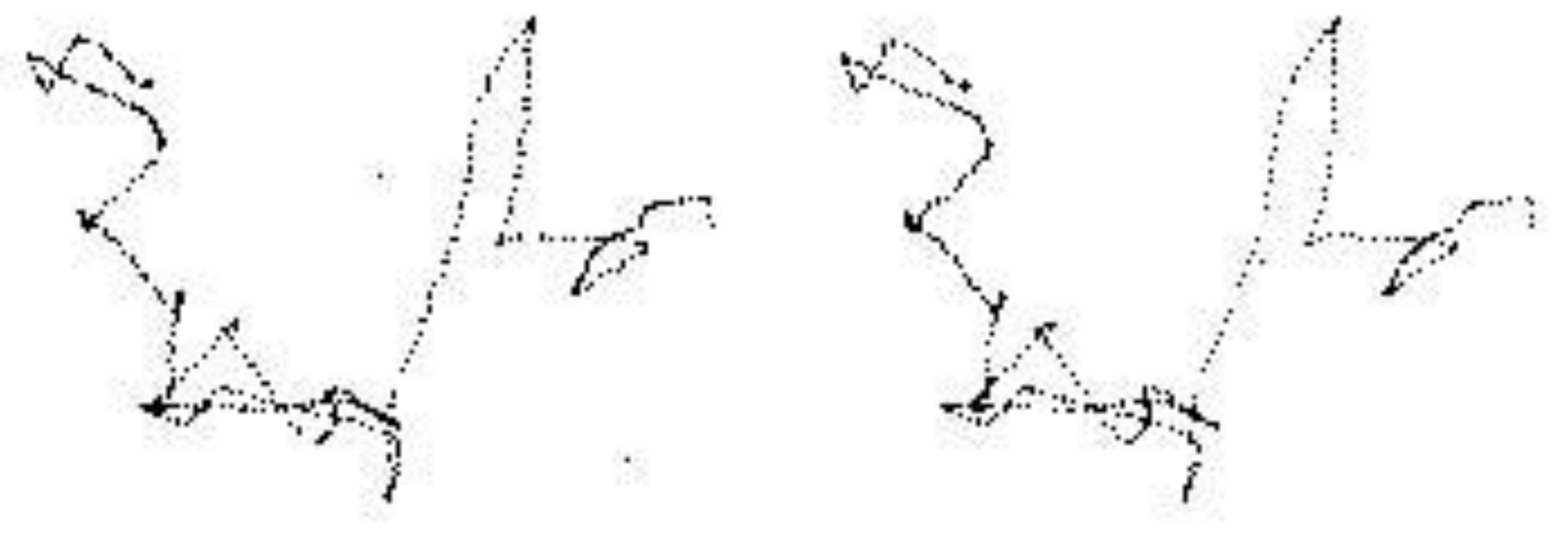
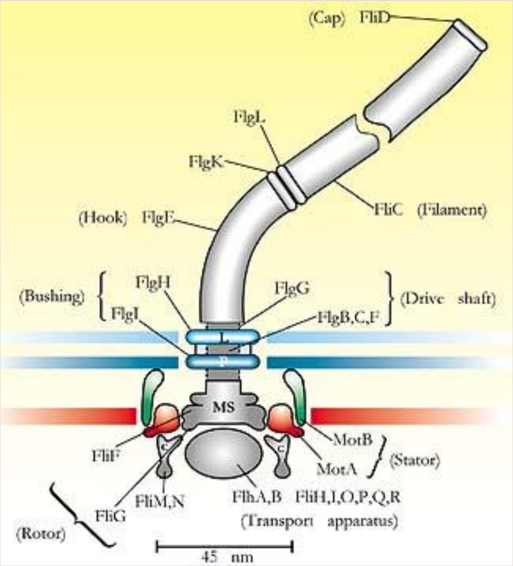


Fluorescent flagellar filaments of *E. coli.*

Random walk by E. coli

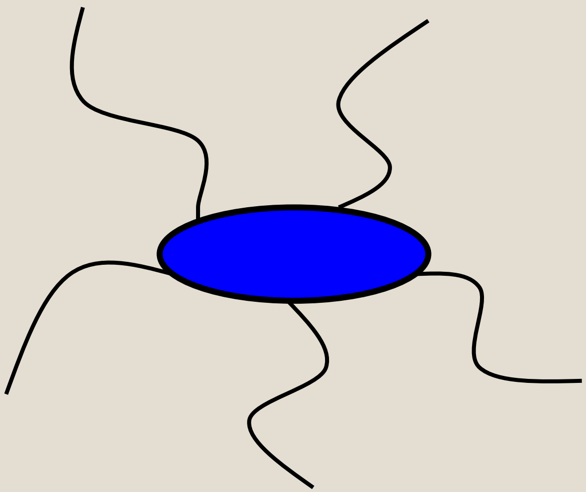


Berg, Physics Today, “Motile behavior of bacteria” (http://www.aip.org/pt/jan0

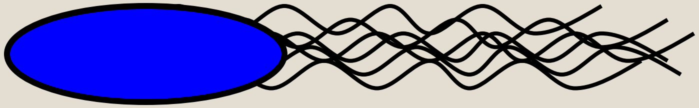


Clockwise

Counter-clockwise



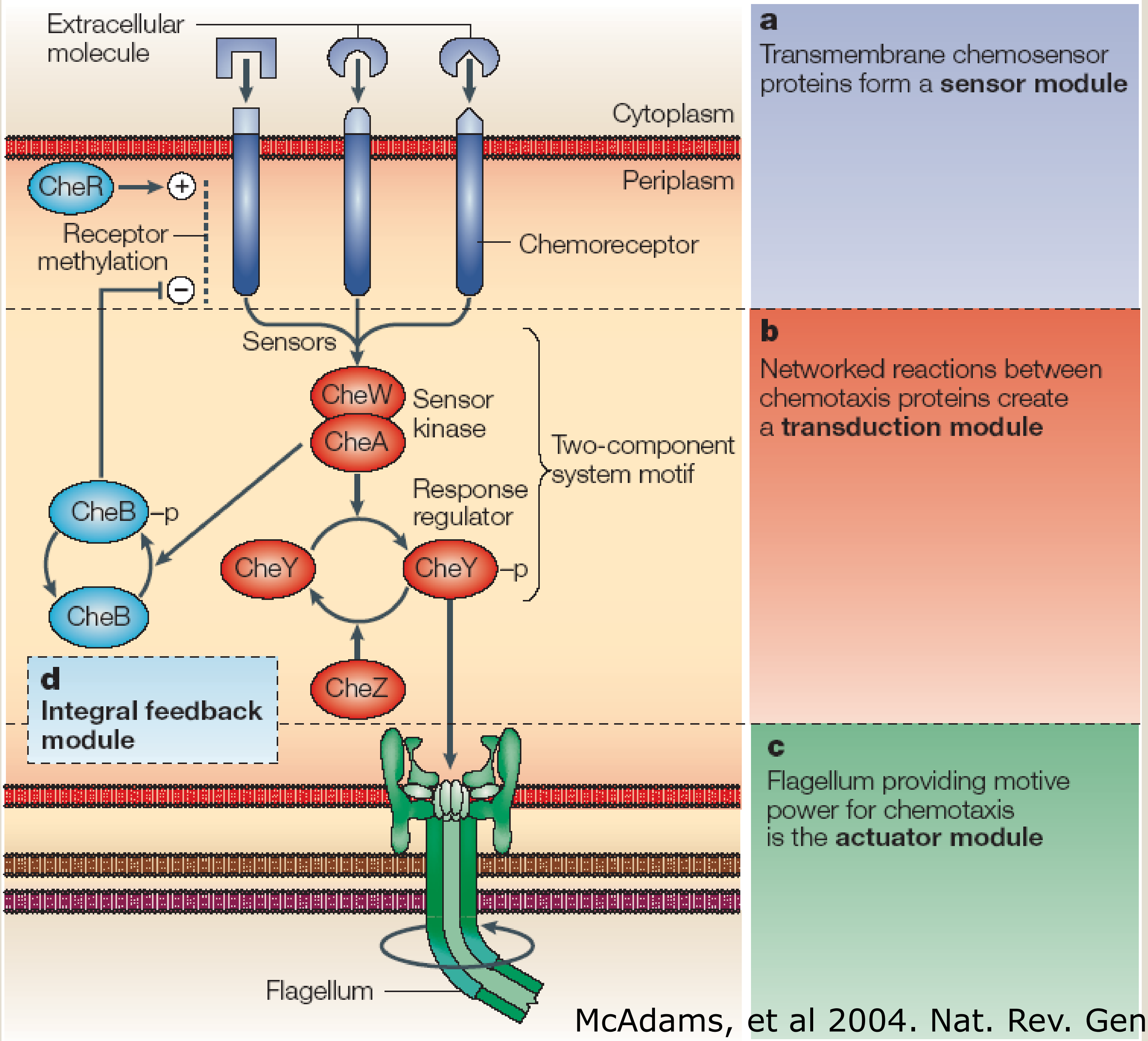
Tumble



Run

Proteins involved in chemotaxis

Protein	**Role**
Receptor	**Senses attractants and repellents**
CheA	Histidine Kinase
CheY	Response Regulator (binds to switch)
CheB	Response Regulator (methylesterase)
CheR	Methylates COOH groups on Receptor (adaptation)
CheZ	Phosphatase (Hydrolyzes CheY-P)



a
Transmembrane chemosensor proteins form a **sensor module**

b
Networked reactions between chemotaxis proteins create a **transduction module**

c
Flagellum providing motive power for chemotaxis is the **actuator module**

d
Integral feedback module

McAdams, et al 2004. Nat. Rev. Genetics

MCP= methyl accepting chemotaxis proteins

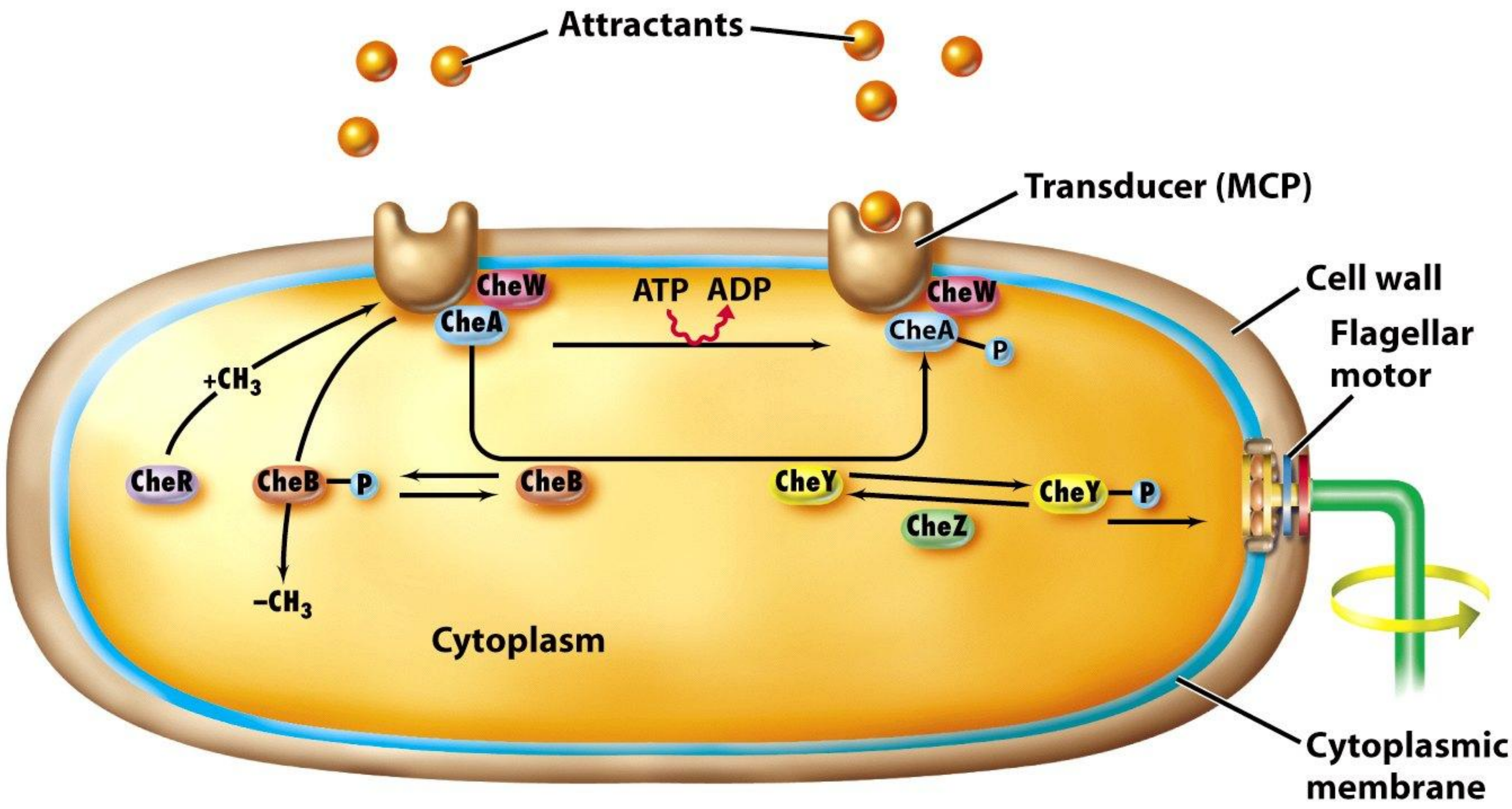


Figure 8-27 Brock Biology of Microorganisms 11/e

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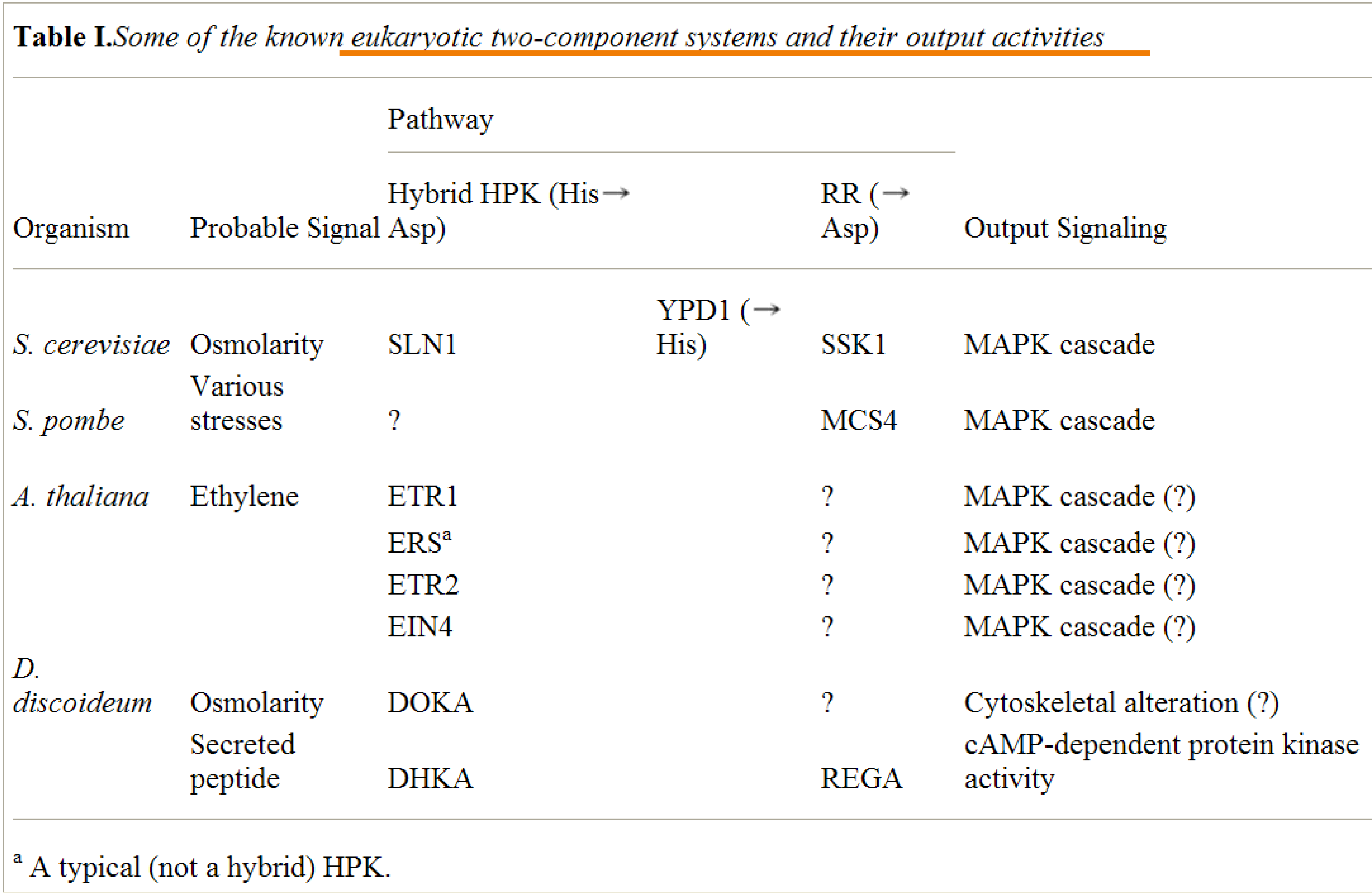
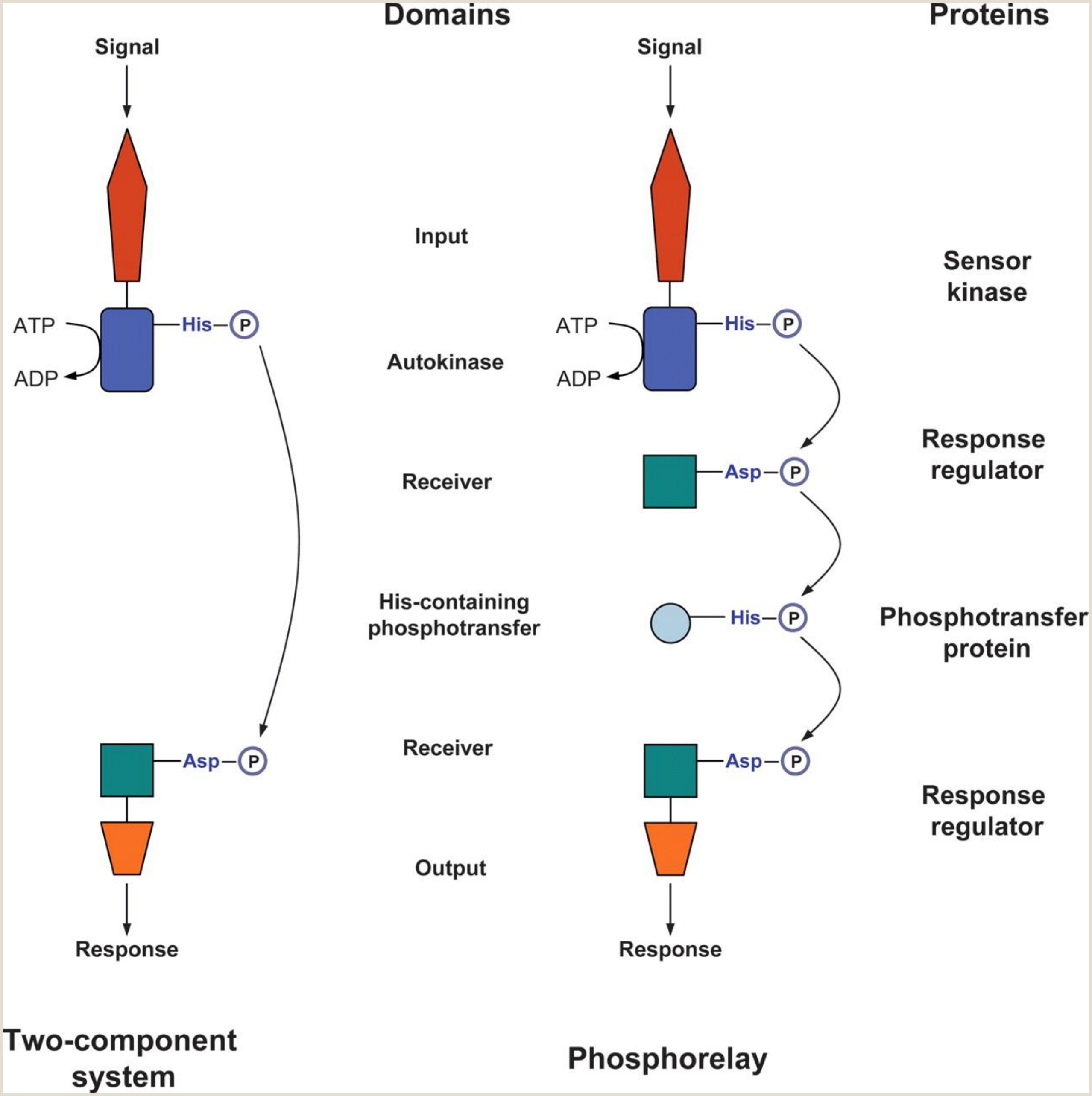


Table I.*Some of the known eukaryotic two-component systems and their output activities*

		Pathway			
Organism	Probable Signal	Hybrid HPK (His→ Asp)		RR (→ Asp)	Output Signaling
S. cerevisiae	Osmolarity	SLN1	YPD1 (→ His)	SSK1	MAPK cascade
S. pombe	Various stresses	?		MCS4	MAPK cascade
A. thaliana	Ethylene	ETR1		?	MAPK cascade (?)
		ERS[a]		?	MAPK cascade (?)
		ETR2		?	MAPK cascade (?)
		EIN4		?	MAPK cascade (?)
D. discoideum	Osmolarity	DOKA		?	Cytoskeletal alteration (?)
	Secreted peptide	DHKA		REGA	cAMP-dependent protein kinase activity

[a] A typical (not a hybrid) HPK.

Schematics of the proteins and domains that constitute TCSs (left) and phosphorelays (right).



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Mitrophanov A Y , Groisman E A Genes Dev. 2008;22:2601-2611

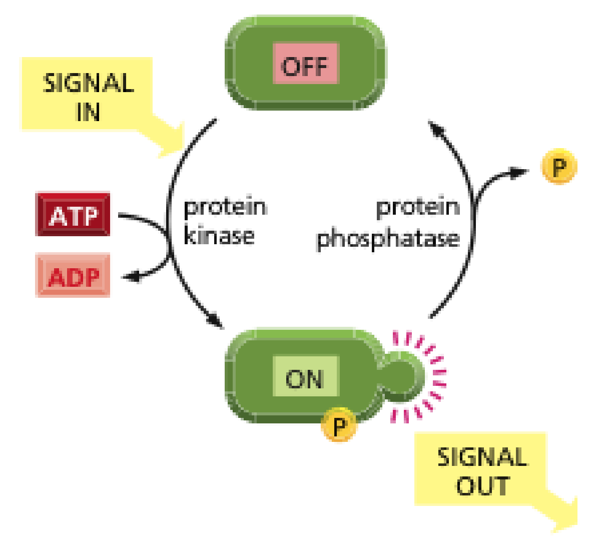
Transmitting the Signal: Protein Kinases

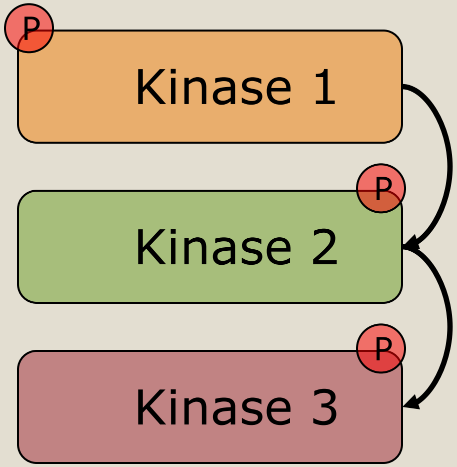
- Protein kinases are enzymes that add a phosphate group from ATP onto a substrate protein; this reaction is called phosphorylation

- Phosphorylation frequently serves to activate the substrate of the kinase, but can also target the substrate for degradation

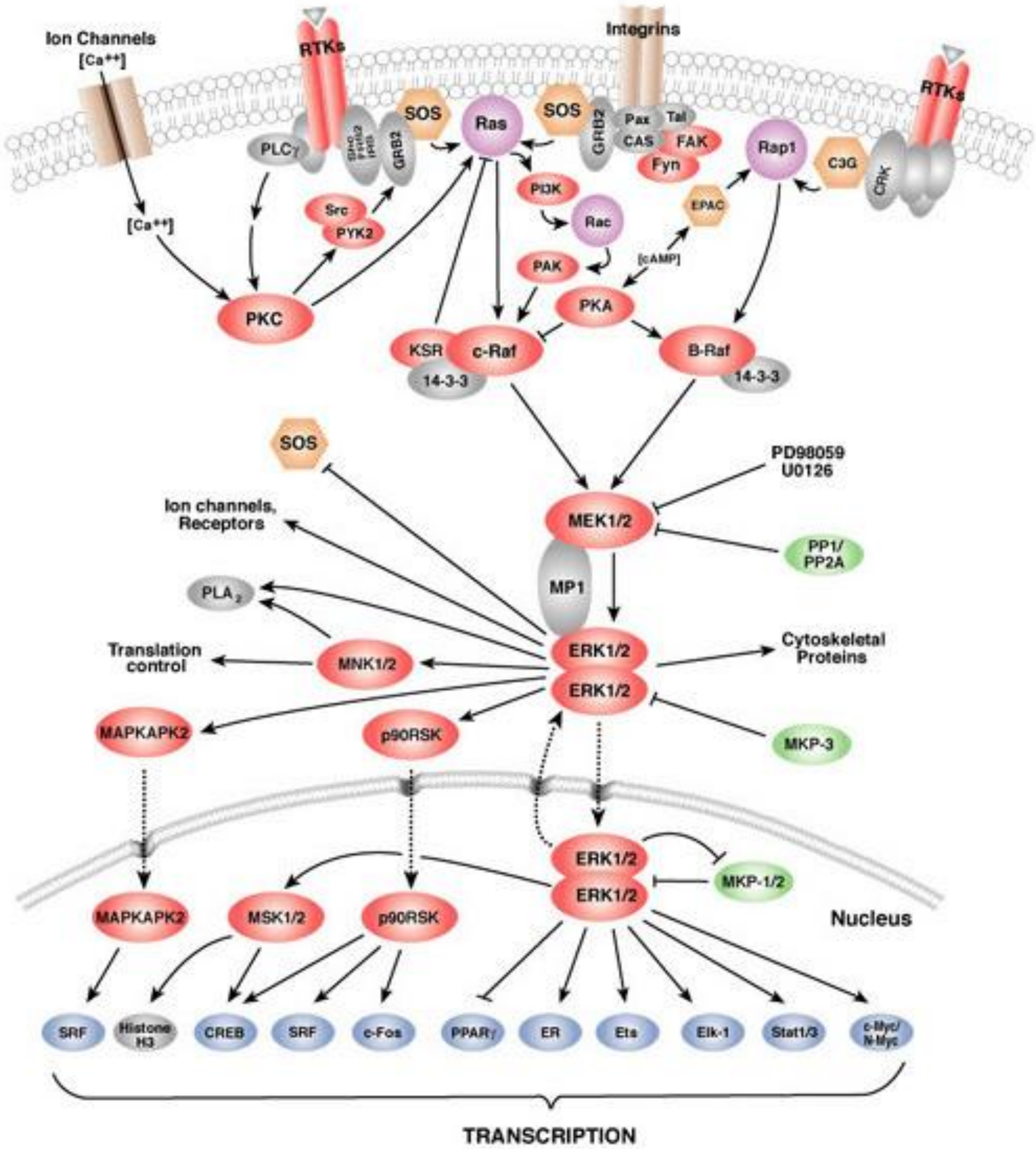
- Kinases are often themselves activated by other kinases via phosphorylation and can organize into phosphorylation cascades

- One important class of phosphorylation cascade is called a mitogen activated protein kinase (MAPK) cascade




P
Kinase 1
P
Kinase 2
P
Kinase 3

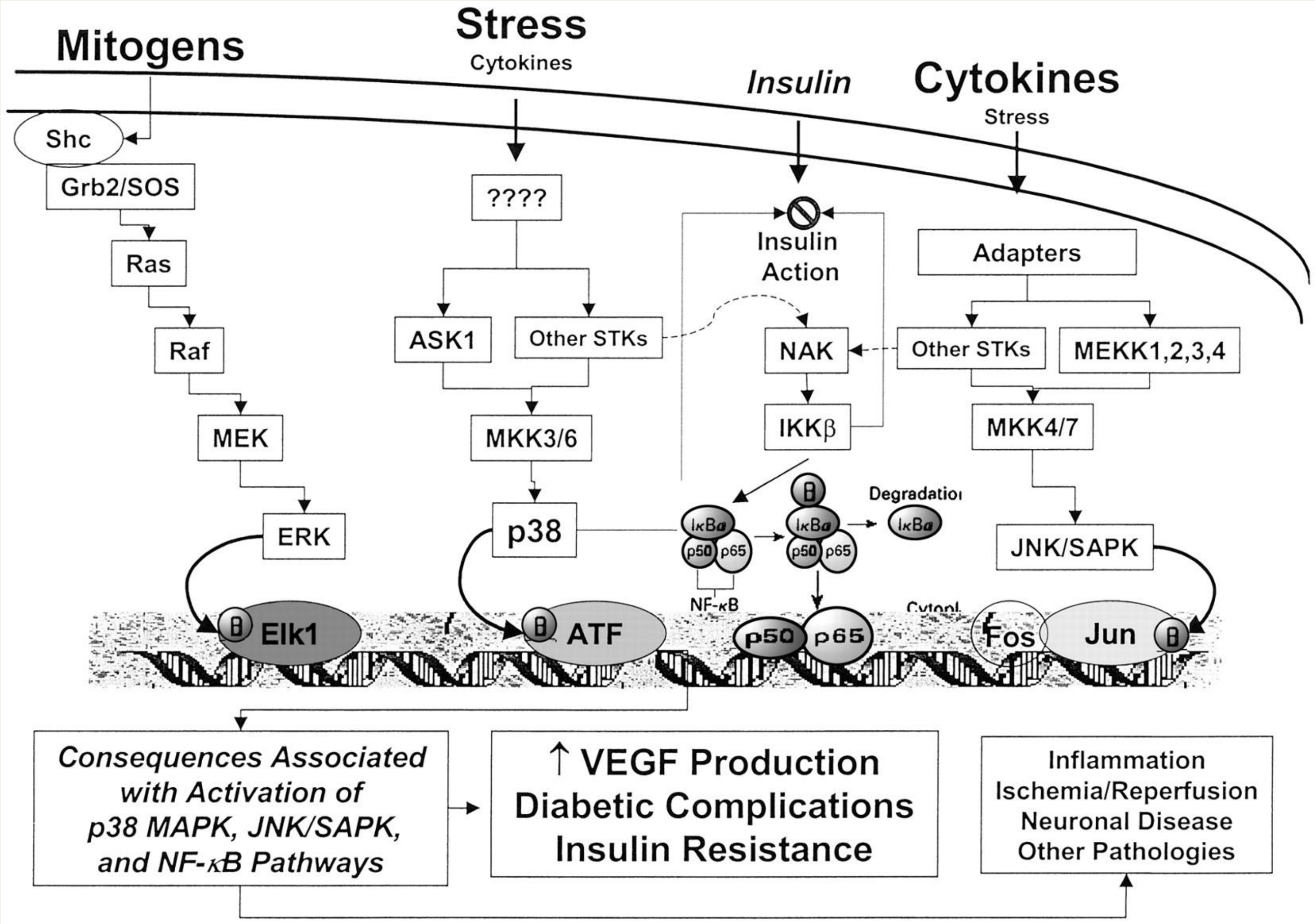
Phosphorylation Cascade



Signaling cascades have many steps

- ❑Modules
- ❑Circuits/ Design Logic
- ❑Cross-talk
- ❑Specificity

Overview of the MAPK superfamily.



Evans J L et al. Endocrine Reviews 2002;23:599-622

ENDOCRINE REVIEWS

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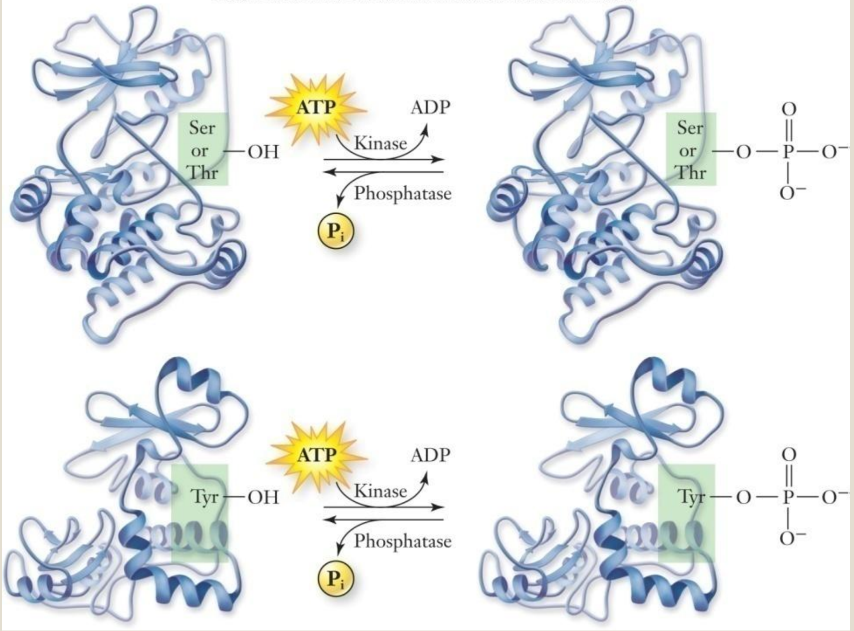
Signal Pathways

- Second messengers are not restricted to a single cell type
- The same second messenger in one cell type can have dramatically different effects in another cell type

Protein modification is the name of the game

- Phosphorylation and dephosphorylation
  - Activates or deactivates proteins (enzymes)
  - Kinases add phosphate groups
  - Phosphatases remove them
- Serine/threonine
- Tyrosine
  - Have –OH groups and can react with phosphate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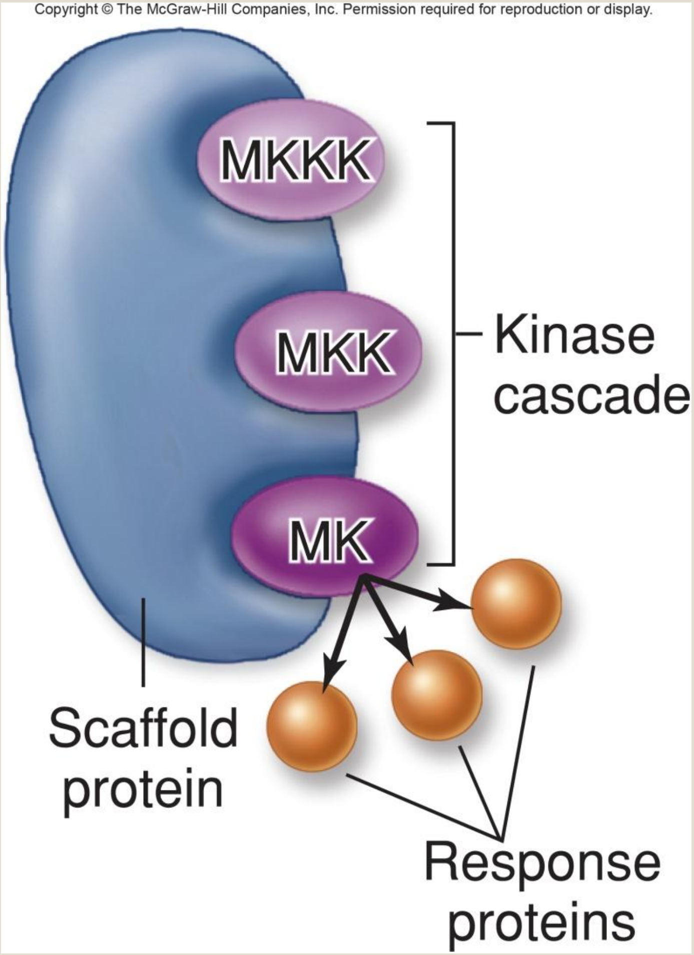
Scaffold proteins

- Organize responses
- Limited by availability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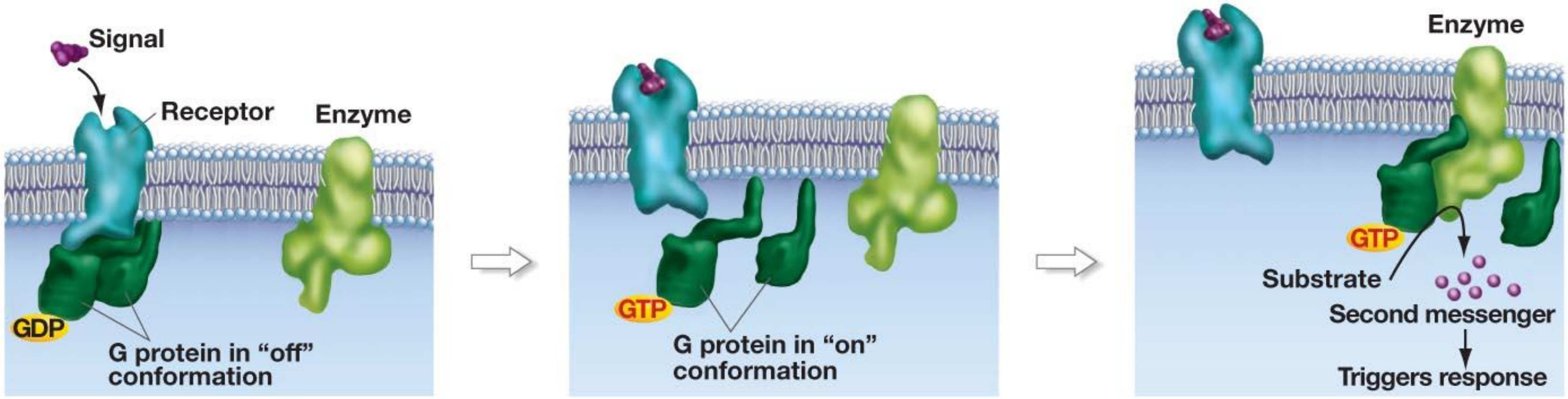
G-Protein

HOW DO G PROTEINS WORK?



1. G protein binds GDP. Signal arrives and binds to receptor.

2. Signal-receptor complex changes conformation. G protein binds GTP and splits into two parts.

3. In response to binding of activated G protein, enzyme catalyzes a reaction that produces a second messenger.

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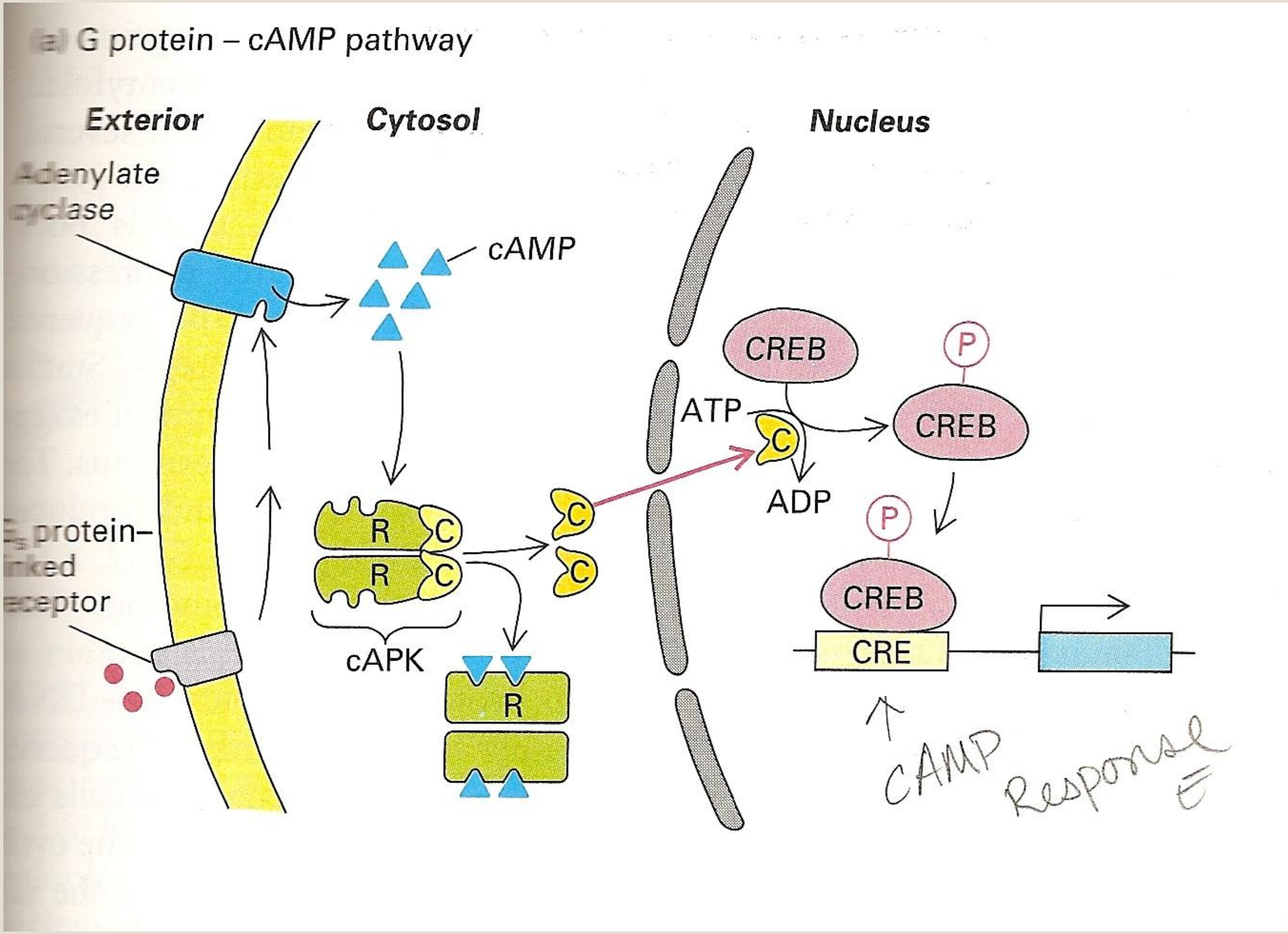
G-Proteins

- Often lead to the activation of protein kinases
- Signal is turned off by enzymes which remove GTP
- Kinases are enzyme which put phosphate groups on proteins of other enzymes
- Phosphate groups have dramatic effects on proteins or enzymes
  - Activation or deactivation of enzymes
  - Conformational shape changes

G-Proteins


G protein – cAMP pathway
Exterior
Cytosol
Nucleus
denylate
yclase
cAMP
protein–
nked
eceptor
R
C
R
C
cAPK
C
C
R
ATP
C
ADP
CREB
P
CREB
P
CREB
CRE
cAMP Response E

Ras is a G protein that helps regulate cell growth

- Ras is inactive when bound to GDP, but activated when bound to GTP
- Ras interacts with RTKs whose ligands are growth factors
- When Ras is activated it will trigger the MAP kinase cascade
- Ras-GTP will inactivate itself by forming GDP and Pi

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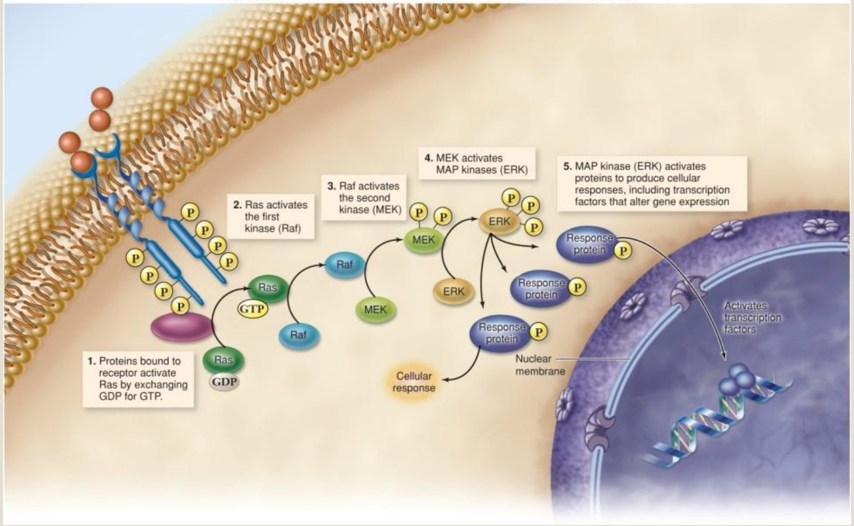


1. Proteins bound to receptor activate Ras by exchanging GDP for GTP.

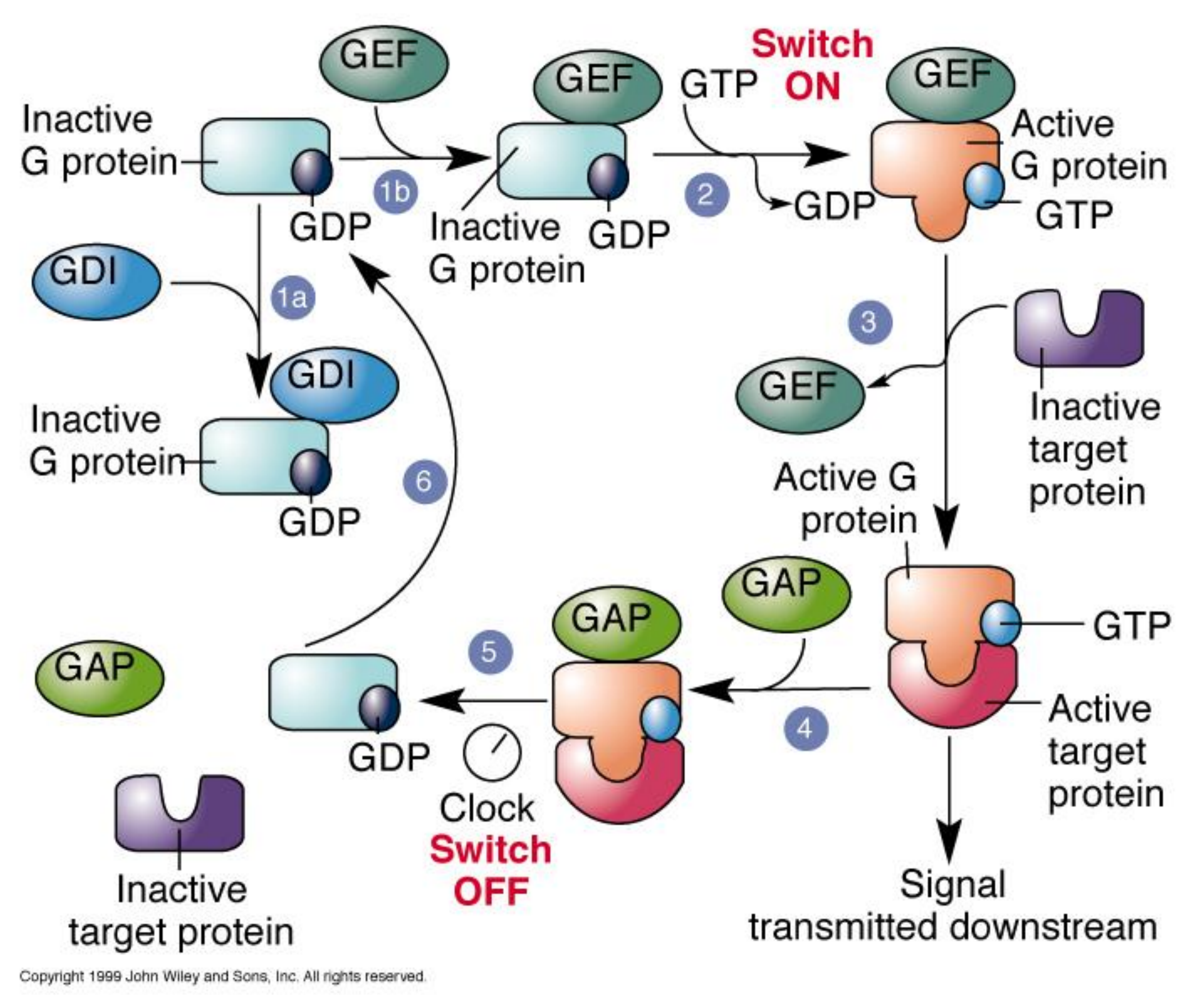
2. Ras activates the first kinase (Raf)

3. Raf activates the second kinase (MEK)

4. MEK activates MAP kinases (ERK)

5. MAP kinase (ERK) activates proteins to produce cellular responses, including transcription factors that alter gene expression

Fig 15.3 The G Protein Cycle



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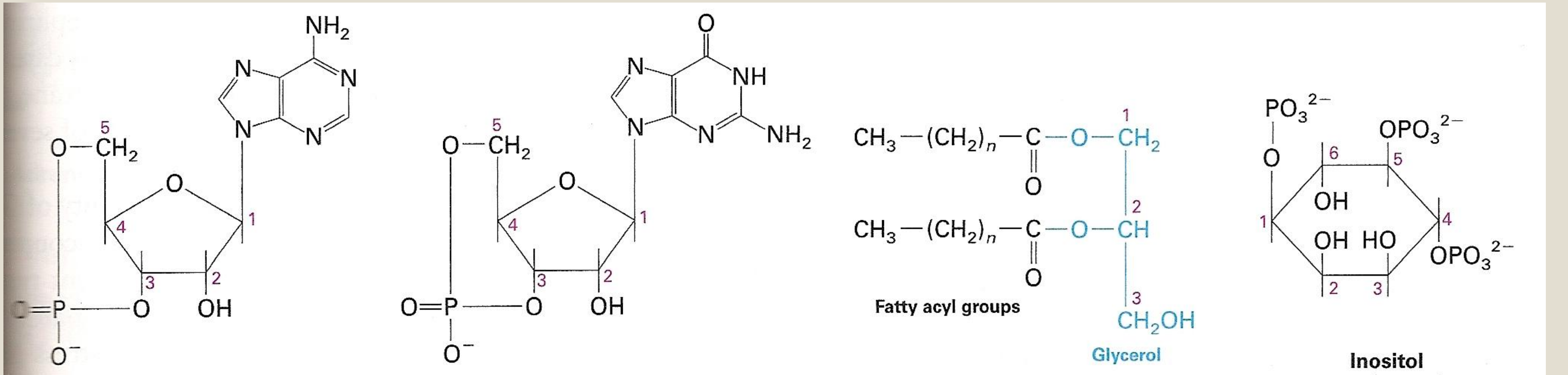
What is a second messenger?

- Effector proteins are often membrane-bound
- Second messenger can move through cell
- Most common examples of effector proteins:
  - Adenylyl cyclase
  - Phospholipase C
  - Can produce many types of second messengers (precursors are already in cell; activated effector proteins change them)

Second Messengers



3′,5′-Cyclic AMP (cAMP)

Activates protein kinase A (PKA)

3′,5′-Cyclic GMP (cGMP)

Activates protein kinase G (PKG) and opens cation channels in rod cells

1,2-Diacylglycerol (DAG)

Activates protein kinase C (PKC)

Inositol 1,4,5-trisphosphate (IP_3)

Opens Ca^{2+} channels in the endoplasmic reticulum

RE 15-9 Four common intracellular second messengers. ajor direct effect or effects of each compound are indicated its structural formula. Calcium ion (Ca^{2+}) and several membrane-bound phosphatidylinositol derivatives also act as second messengers.

Production of second messengers

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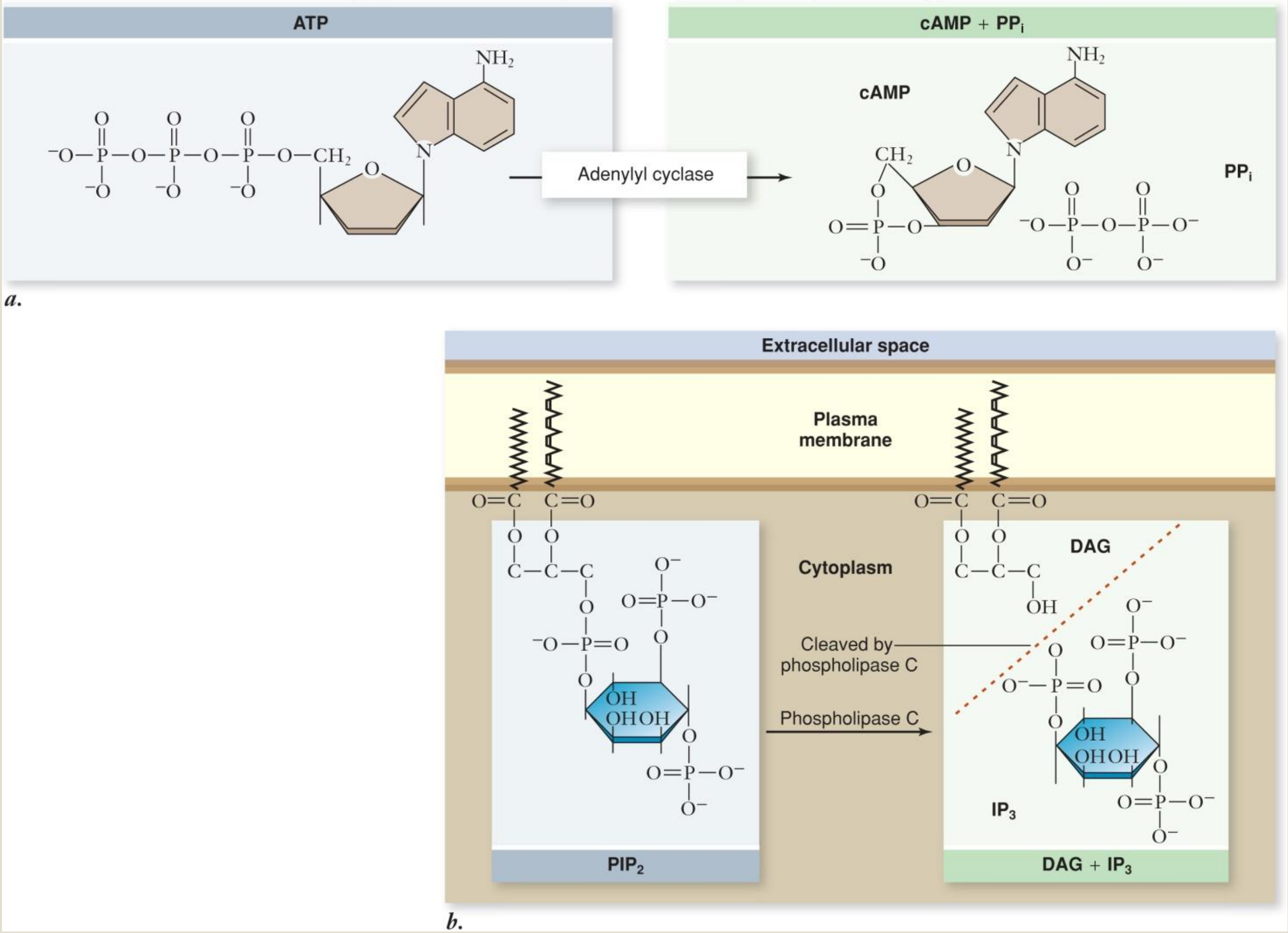

cAMP signaling

- cAMP is quickly inactivated by phospho-diesterase
- Primary target of cAMP is protein kinase A (PKA)
- PKA has 4 subunits: 2 regulatory, 2 catalytic
- cAMP binds to regulatory subunits
- catalytic subunits now detach and areactivated

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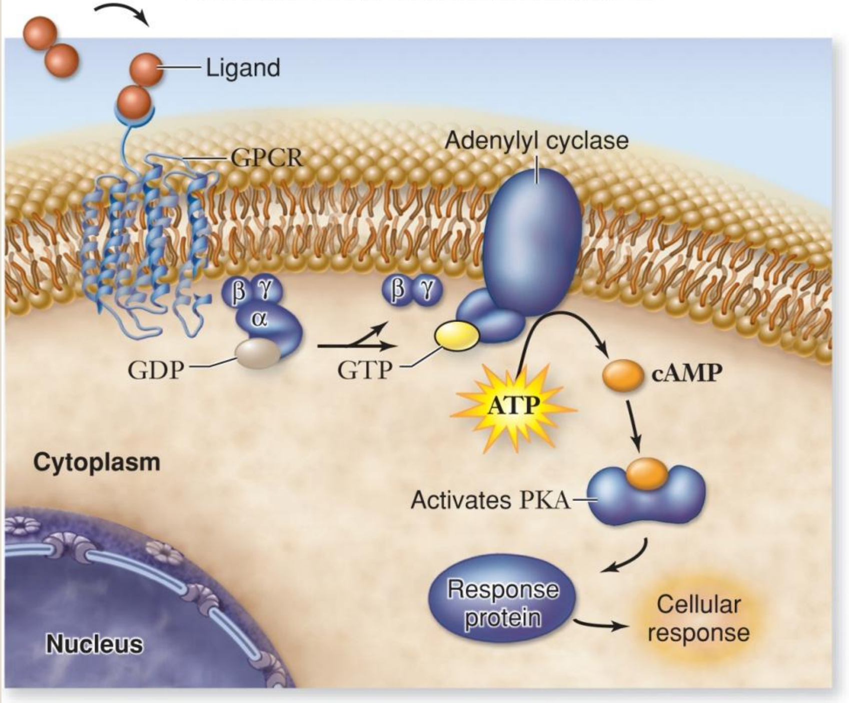

Phosholipase C and second messengers

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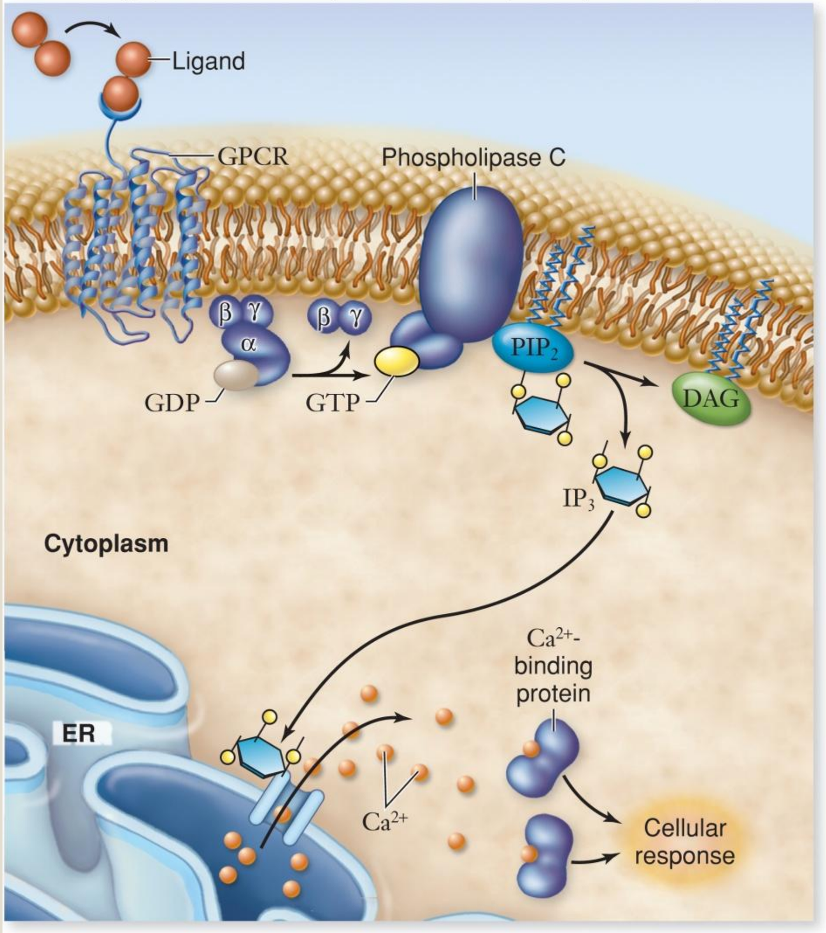
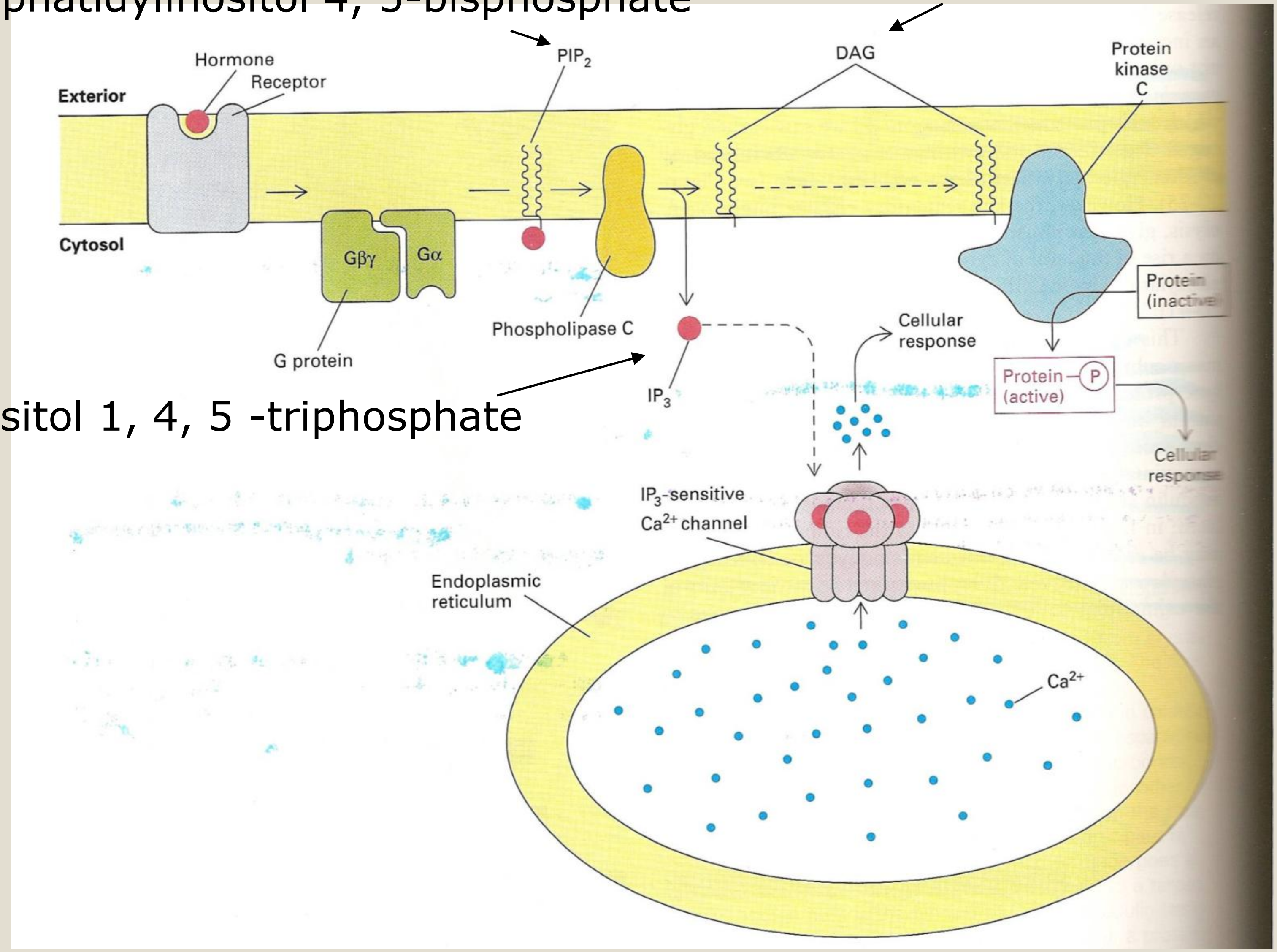


IP_3 and DAG Pathway

Diacylglycerol

Phosphatidylinositol 4, 5-bisphosphate



Inositol 1, 4, 5 -triphosphate

Calcium ions are associated with:

Neurotransmitters
Growth factors
Some hormones

Increase in cytoplasmic concentration of calcium contributes to:

Muscle cell contraction
Neurotransmitter release
Antibody production
Insulin secretion

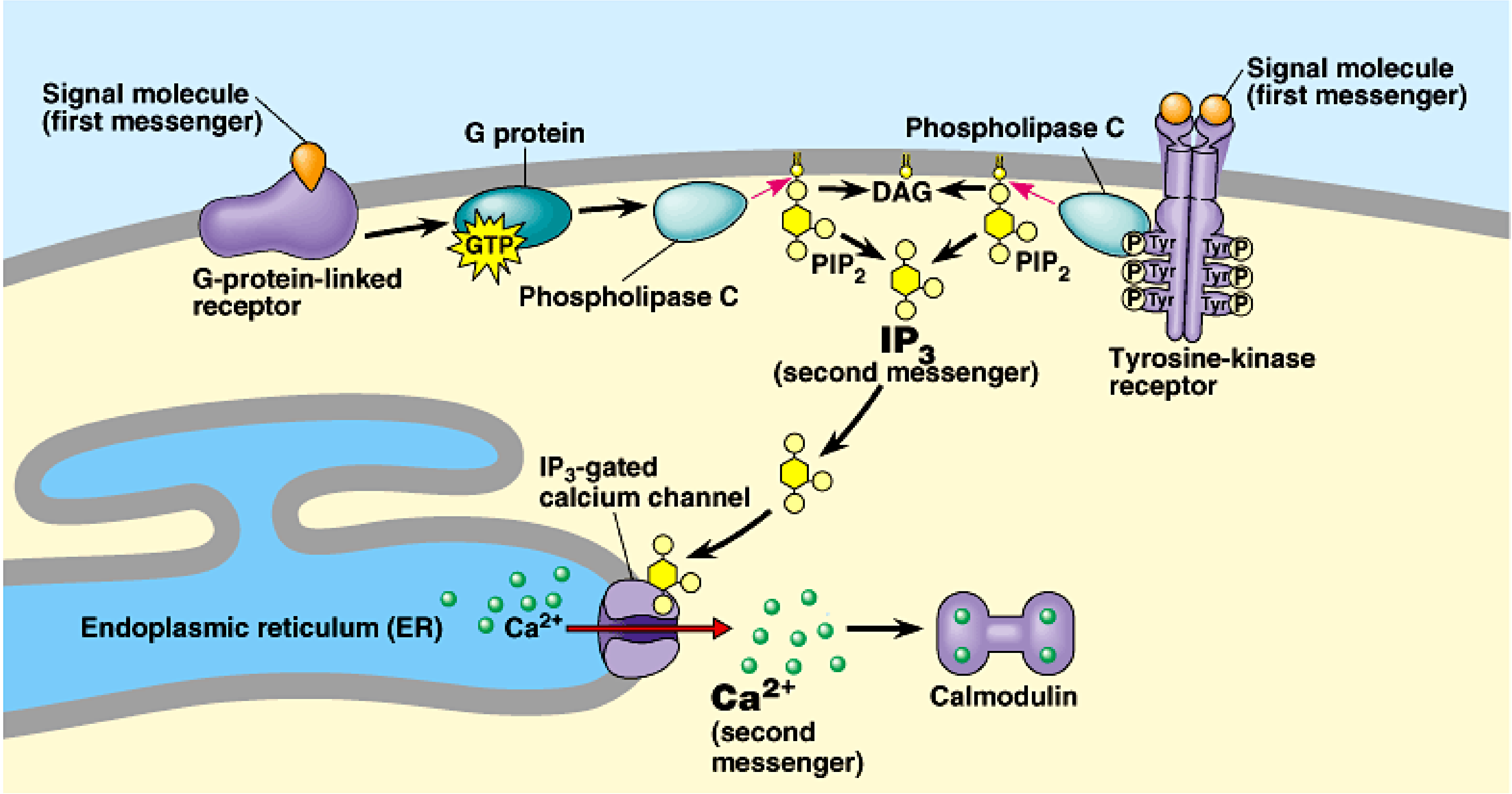
Among others

Both G-protein and tyrosine kinase pathways
can utilize calcium as a second messenger

Calcium concentrations in cytoplasm are normally
very low

Where does the calcium come from?
from the extracellular environment
from the ER

(calcium is exported by calcium pumps within
cell, to keep internal calcium levels low)



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DAG activates protein kinase C (PKC)
phosphorylates many target proteins, at serines or threonines

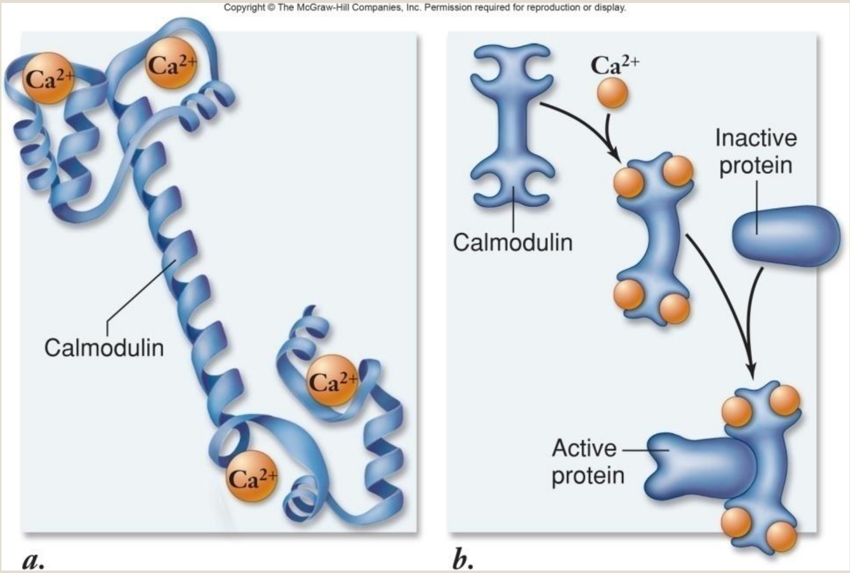
PKC activation (you guessed it) does many things in cells, including:

Cell growth
Activation of ion channels
Protein secretion

Through its ability to phosphorylate kinases associated with growth

Calmodulin functions in many cells

- Neurons
- Smooth muscle cells
- Depending on cell, actives:
  - Kinases
  - Ion channels
  - Receptors
  - And others



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Signal “amplification” in cell proliferation

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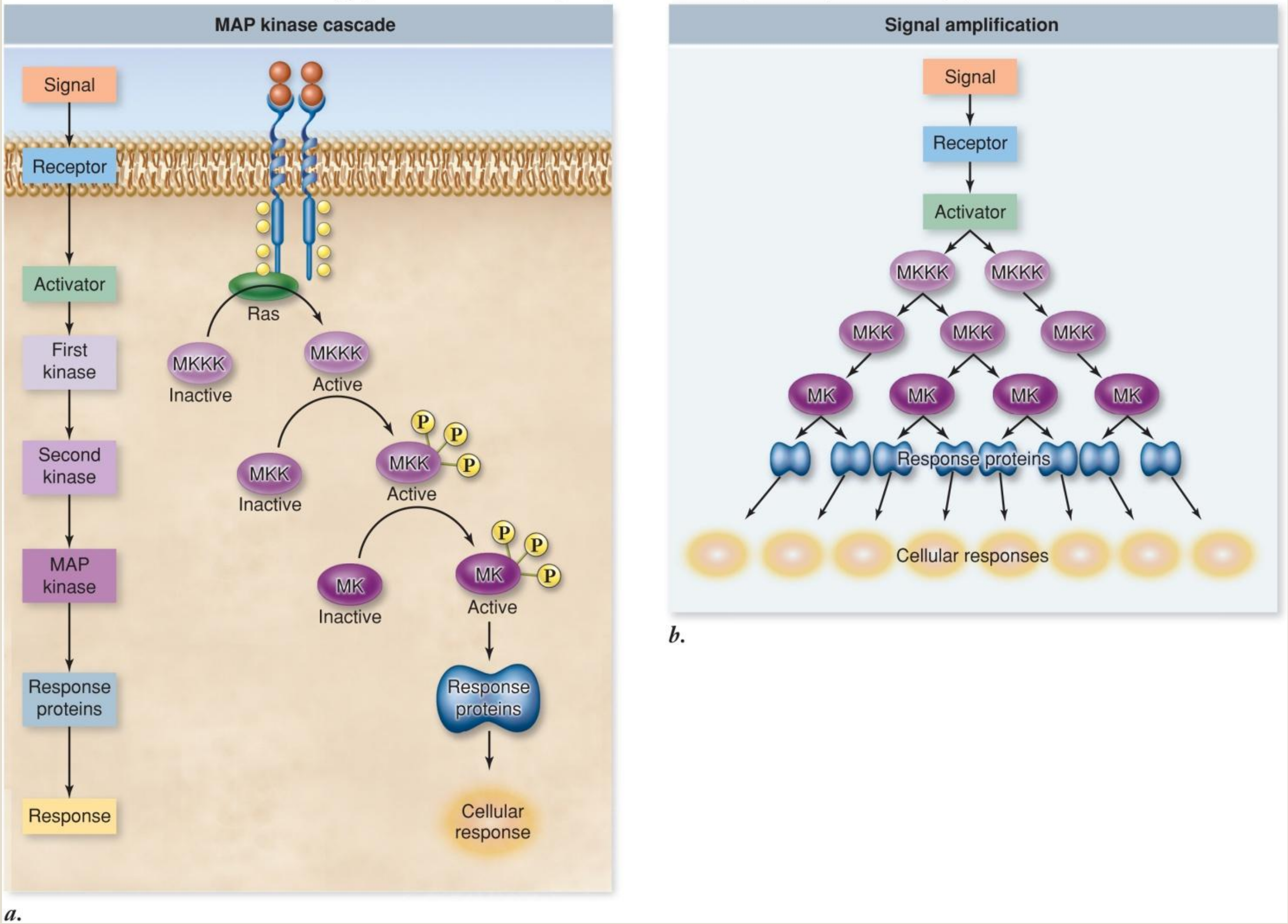

Different receptors can activate a common signaling pathway

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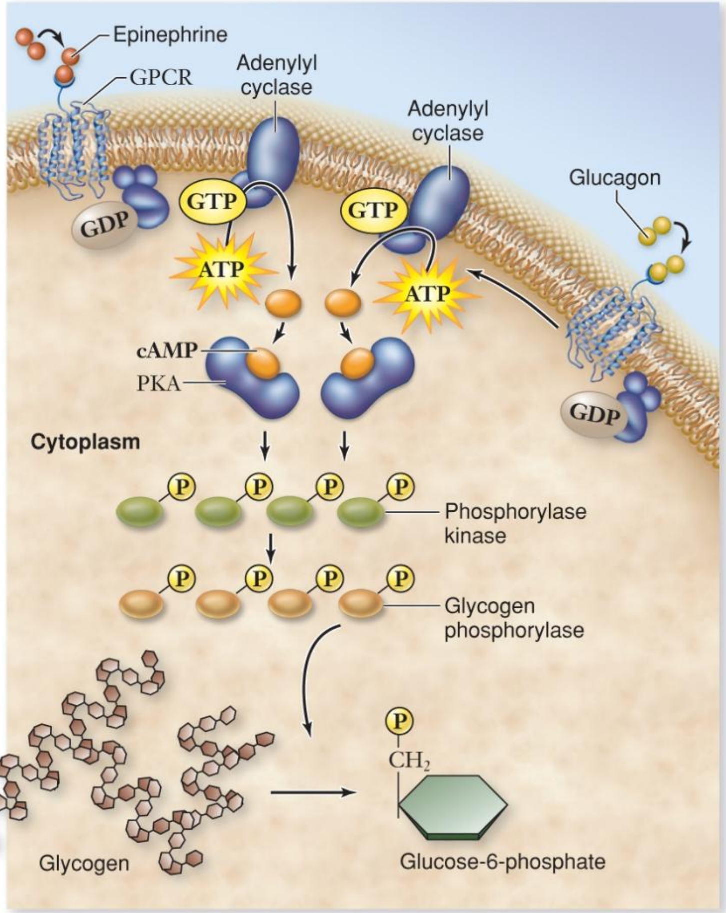


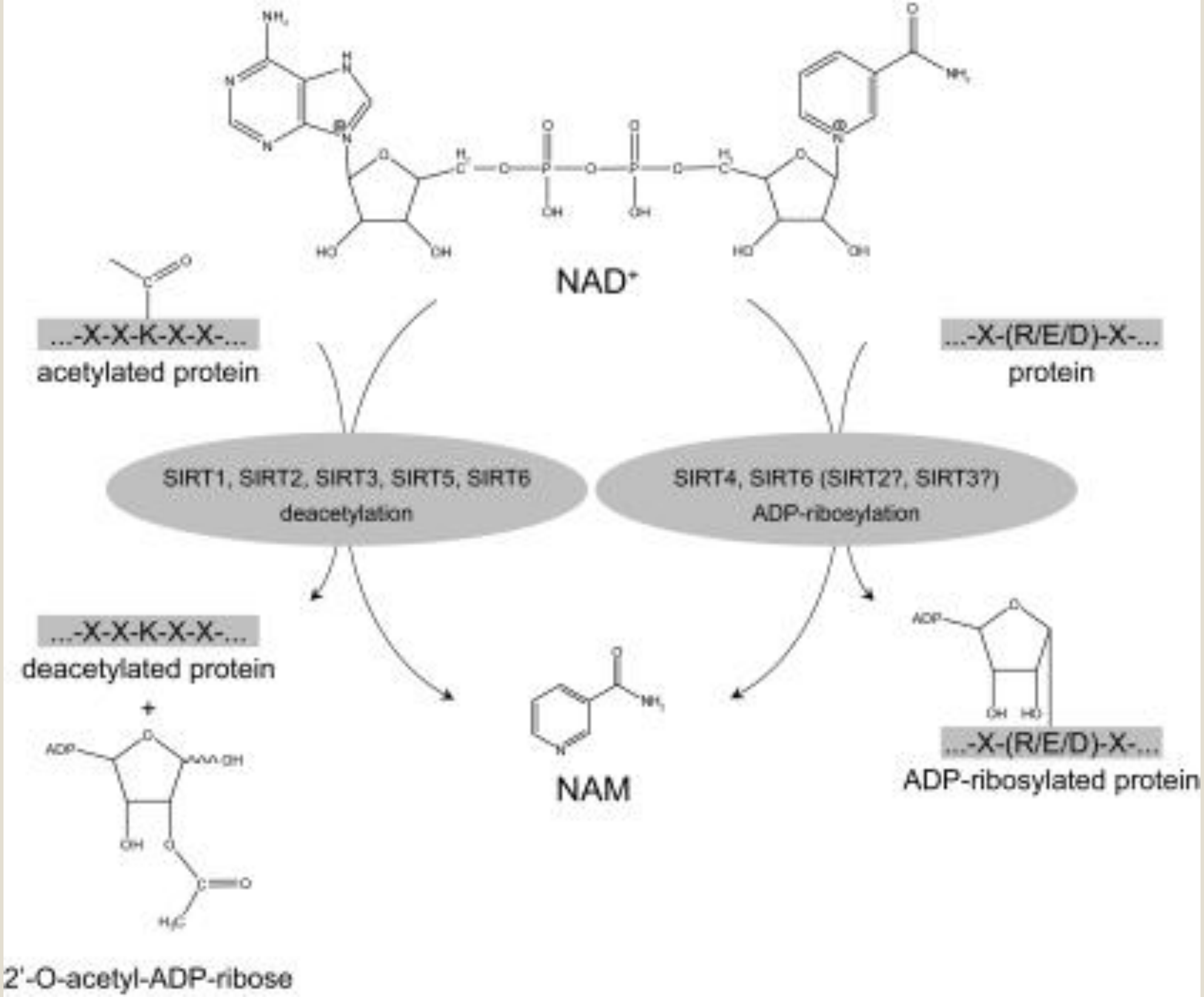
Disruption of G protein signaling and disease

- A tale of two toxins
- Cholera toxin modifies Gs so that it cannot be inactivated
- Pertussis toxin modifies Gi so it cannot inhibit adenylyl cyclase
- Same effect, two different mechanisms, different target cells

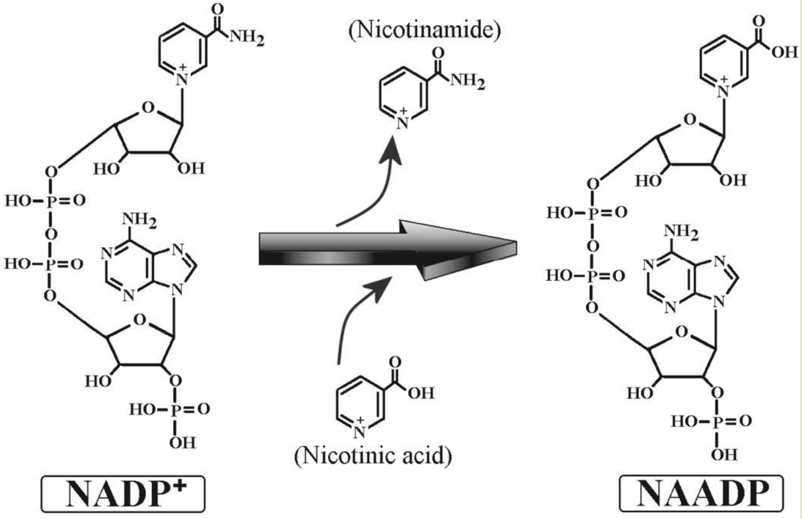
- NAD/NADH signaling
- ATP/ADP signaling
- Metabolic speciation

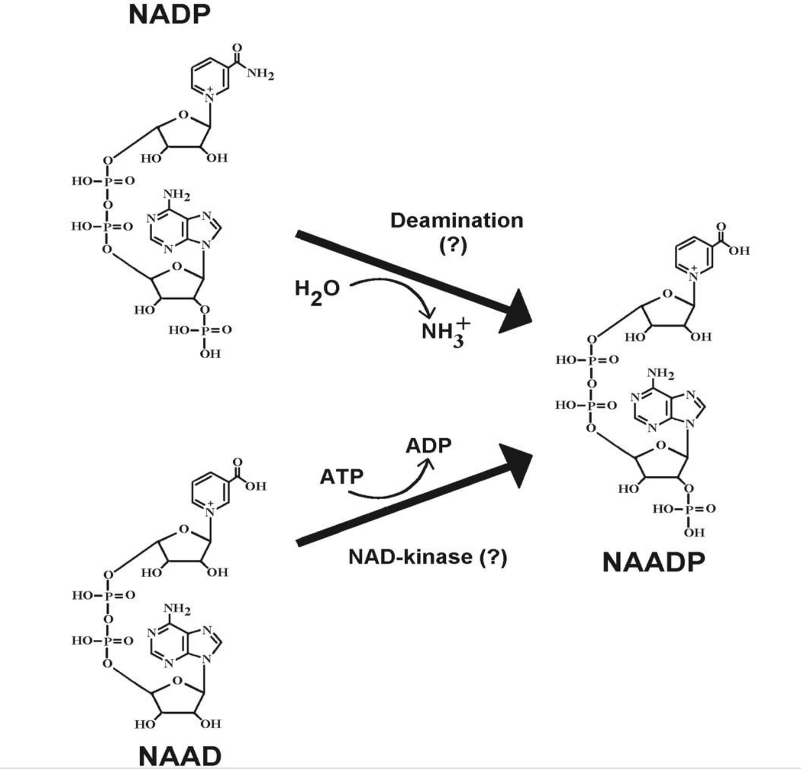
The Secret Life of NAD$^+$: An Old Metabolite Controlling New Metabolic Signaling Pathways

http://edrv.endojournals.org/content/31/2/194.long

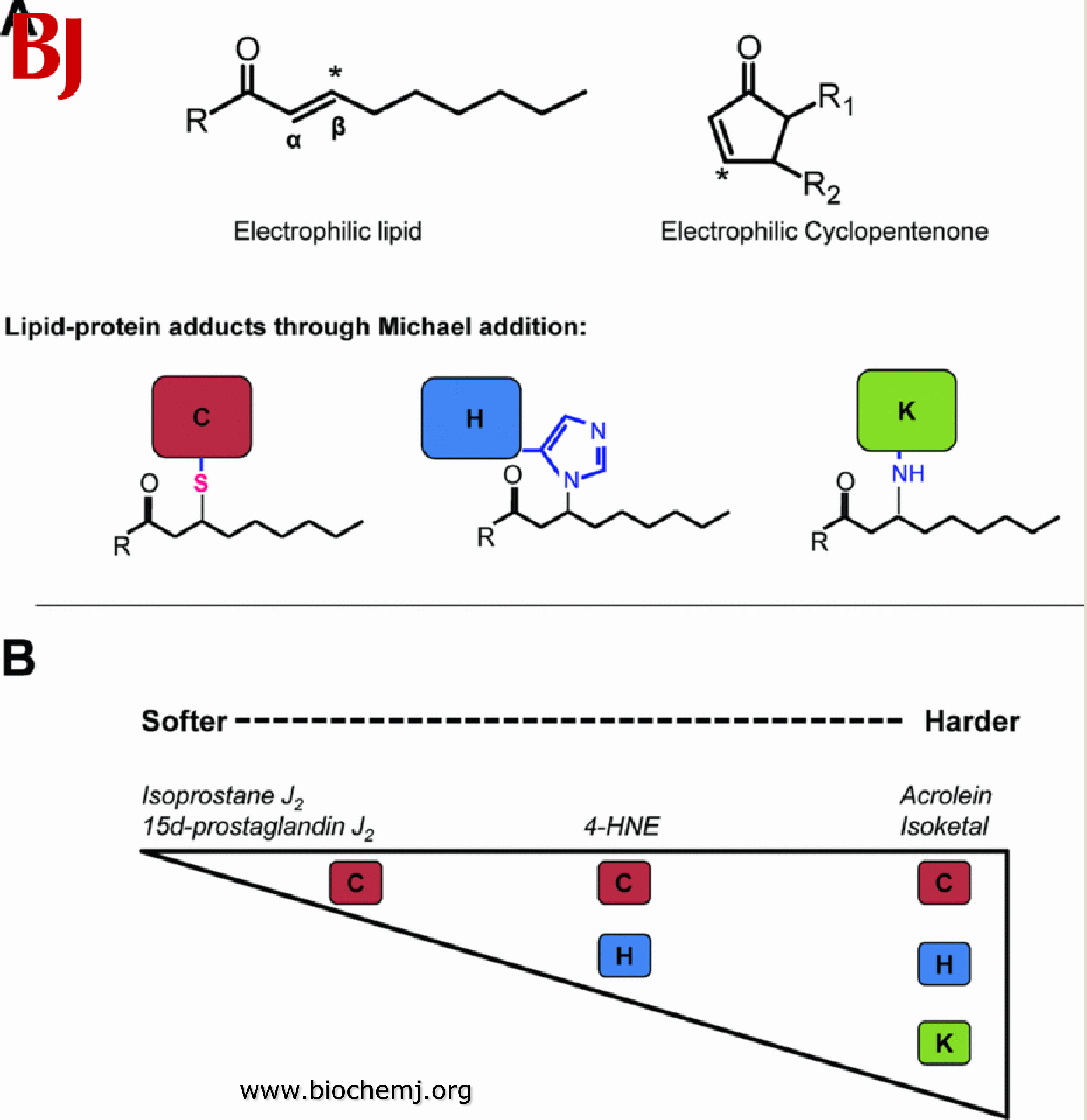


NAADP as a second messenger: neither CD38 nor base-exchange reaction are necessary for in vivo generation of NAADP in myometrial cells



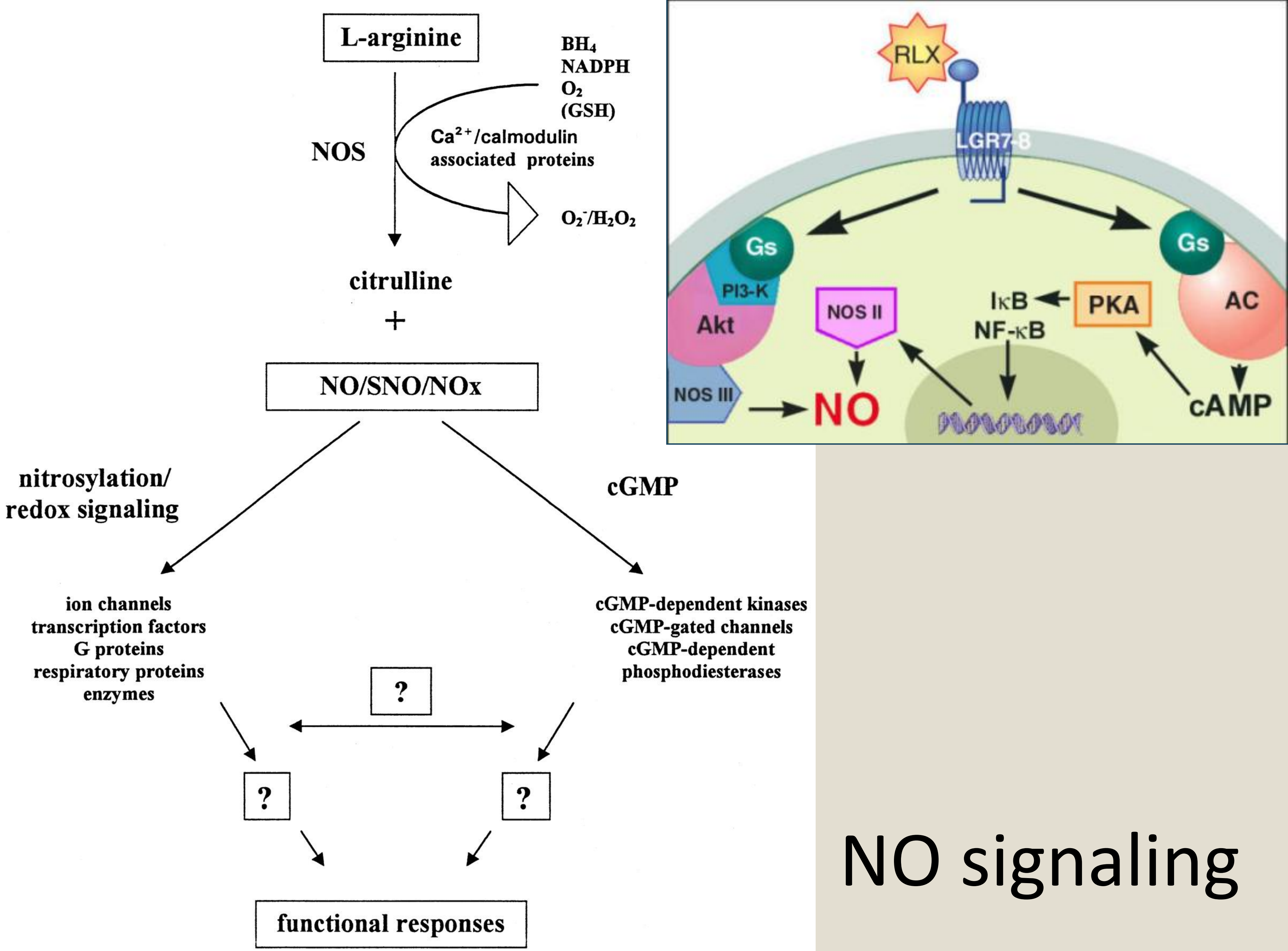


Redox Lipid Species differ in their reactivity with specific amino acids

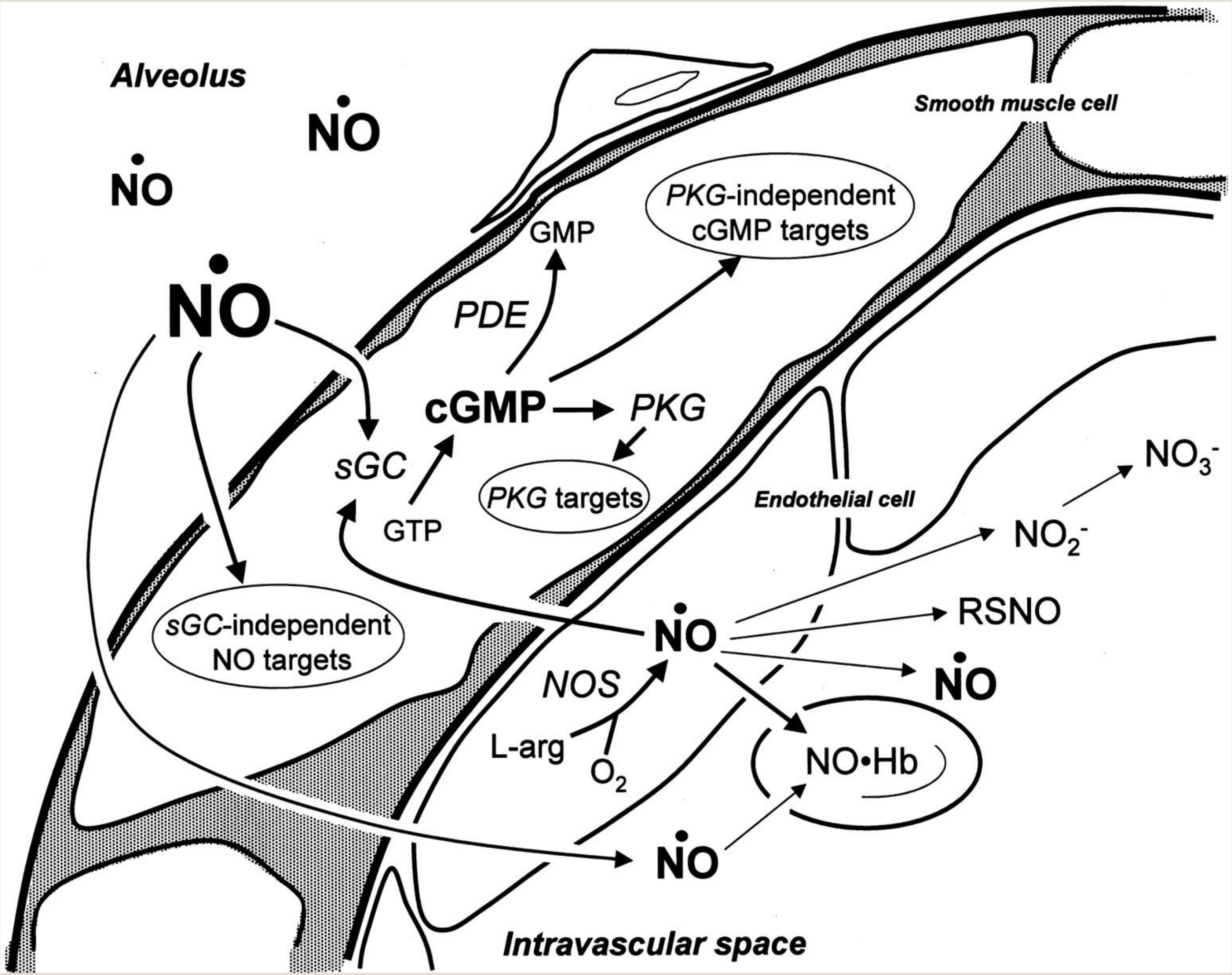

A
O
R
*
α
β
Electrophilic lipid
O
R1
R2
*
Electrophilic Cyclopentenone
Lipid-protein adducts through Michael addition:
C
S
O
R
H
N
N
O
R
K
NH
O
R
B
Softer ------------------------------------ Harder
Isoprostane J2
15d-prostaglandin J2
4-HNE
Acrolein
Isoketal
C
C
H
C
H
K

www.biochemj.org

Biochemical Journal 2012 442, 453-464 - Ashlee Higdon, Anne R. Diers and others.

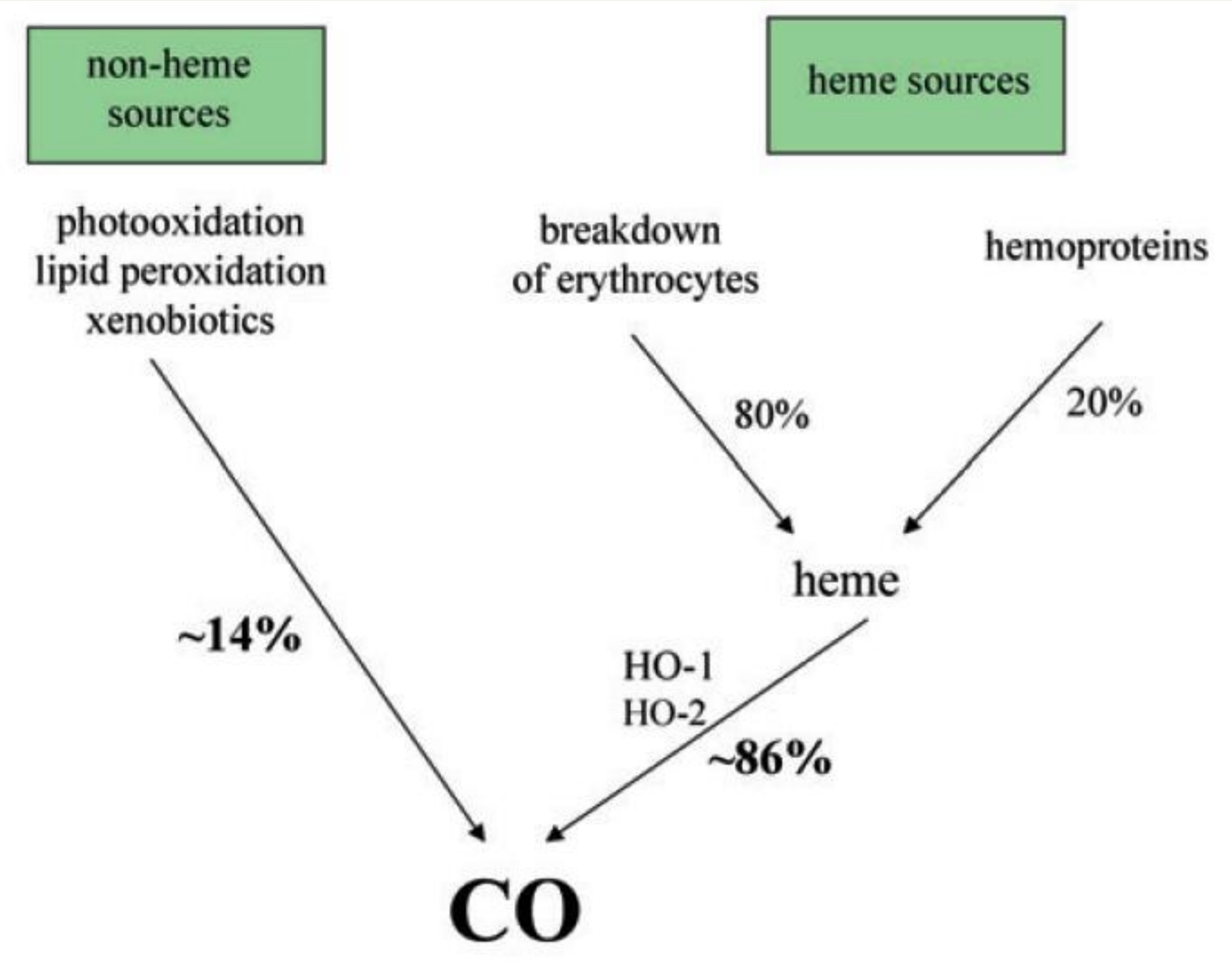


NO signaling

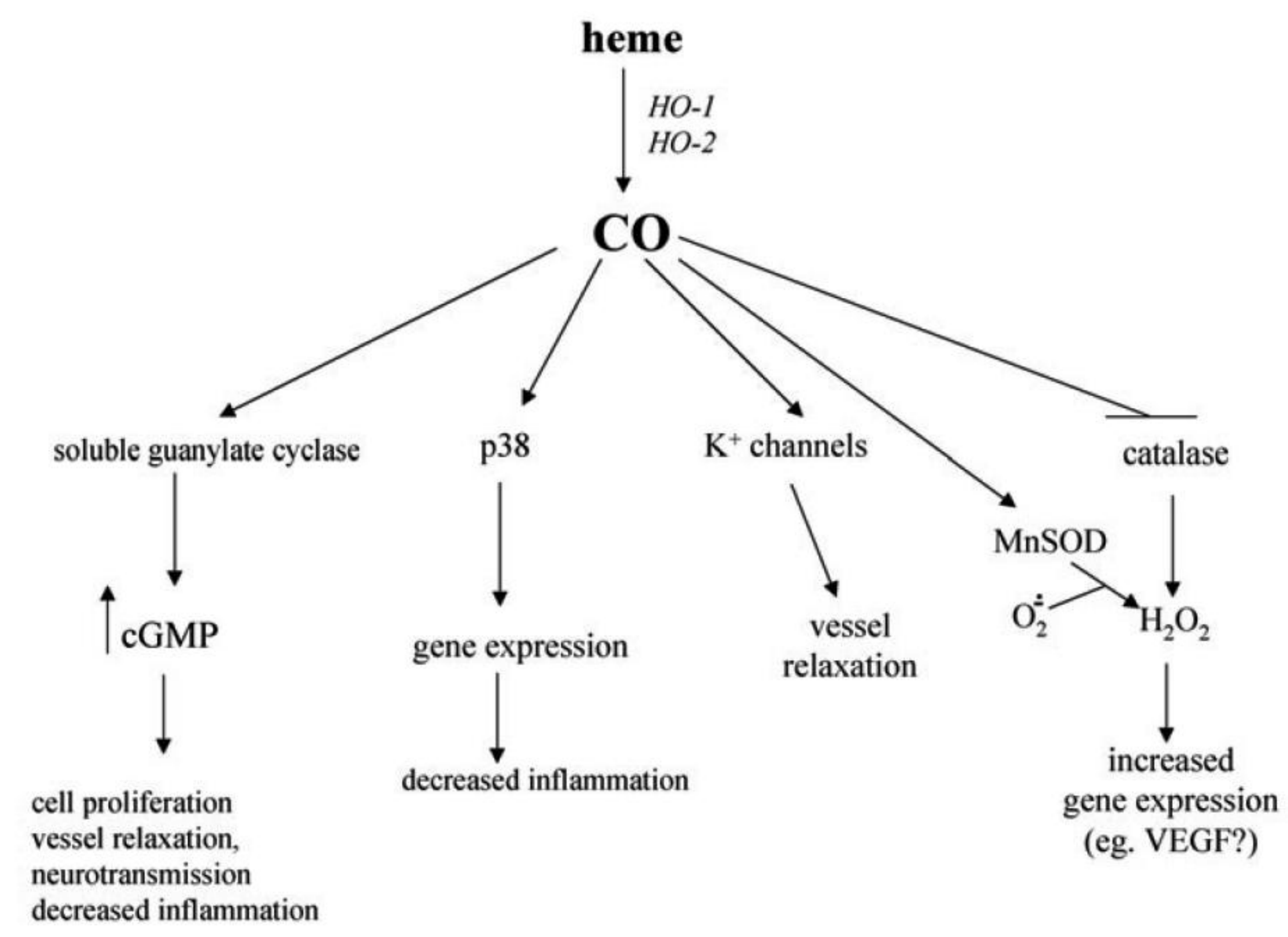


Alveolus
$\dot{N}O$
$\dot{N}O$
$\dot{N}O$
Smooth muscle cell
GMP
PDE
PKG-independent cGMP targets
sGC
cGMP
PKG
GTP
PKG targets
sGC-independent NO targets
Endothelial cell
NO_3^-
NO_2^-
RSNO
$\dot{N}O$
$\dot{N}O$
NOS
L-arg
O_2
NO•Hb
$\dot{N}O$
Intravascular space

Carbon monoxide -- a "new" gaseous modulator of gene expression



The effect of CO on gene expression



heme

HO-1
HO-2

CO

soluble guanylate cyclase

↑ cGMP

cell proliferation
vessel relaxation,
neurotransmission
decreased inflammation

p38

gene expression

decreased inflammation

K^+ channels

vessel relaxation

MnSOD

$O_2^{\cdot-}$

H_2O_2

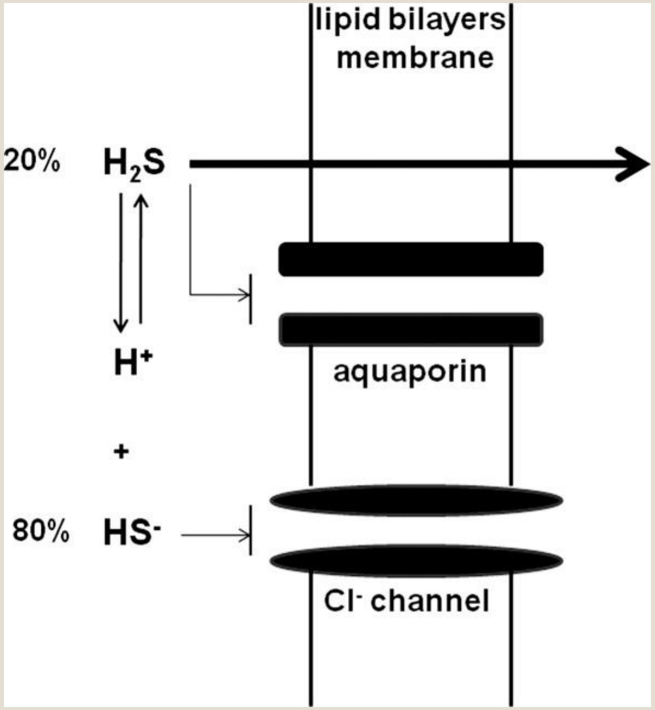
catalase

increased gene expression (eg. VEGF?)

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3342561/

Hydrogen Sulfide Is a Signaling Molecule and a Cytoprotectant

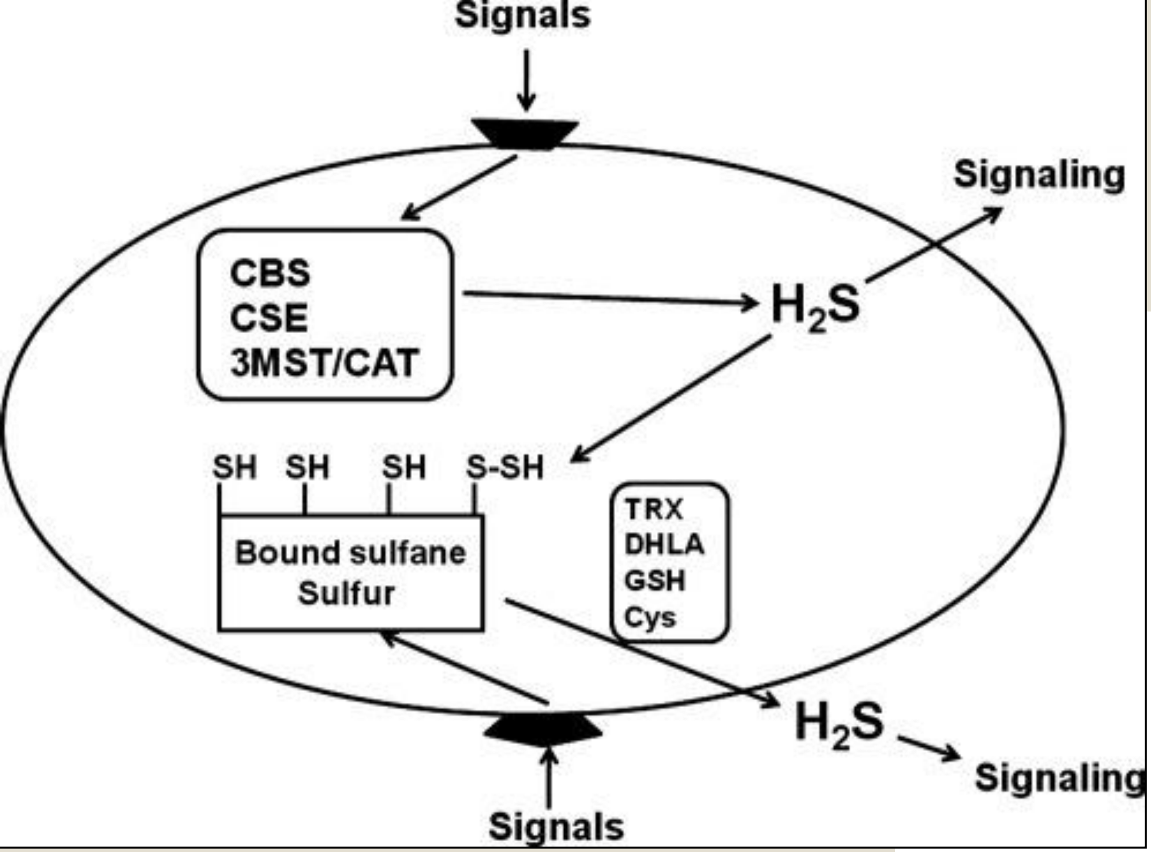
Antioxid Redox Signal. 2012 July 1; 17(1): 45–57

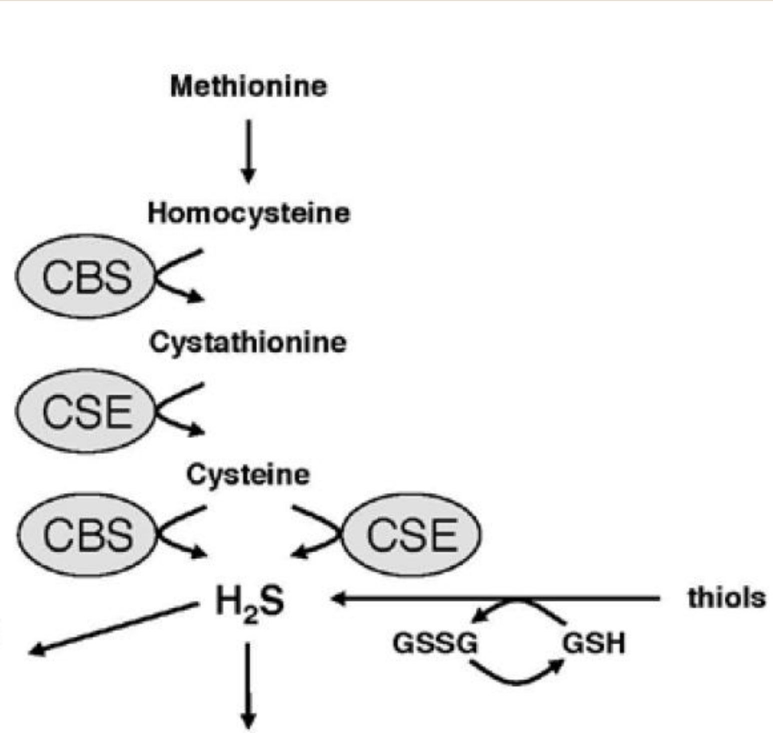


H2S hydrogen sulfide may function as a signaling molecule in processes such as neuromodulation in the brain and smooth muscle relaxation in the vascular system.

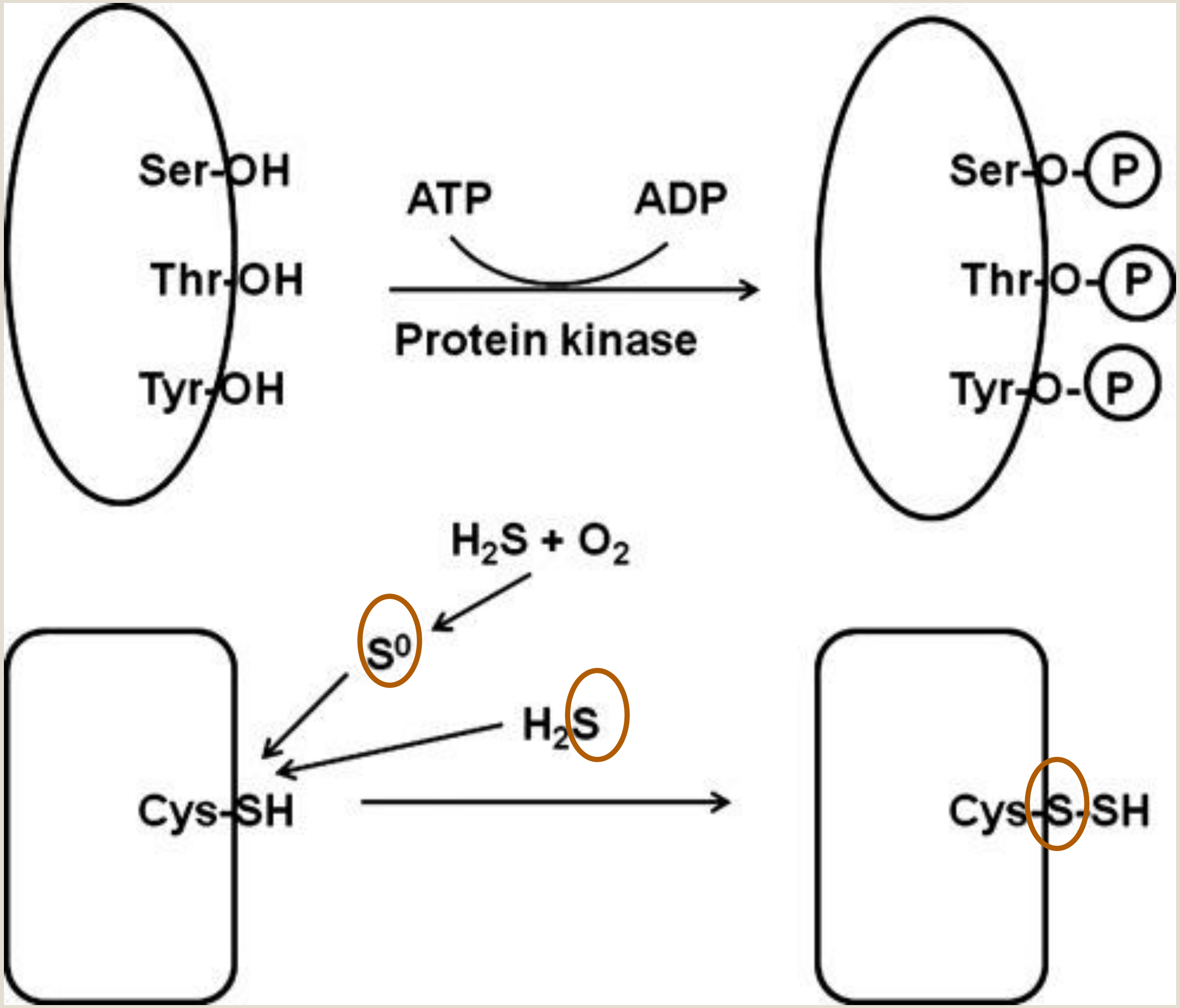
It also has a cytoprotective effect, since it can protect neurons and cardiac muscle from oxidative stress and ischemia-reperfusion injury, respectively.

Hydrogen sulfide can also modulate inflammation, insulin release, and angiogenesis

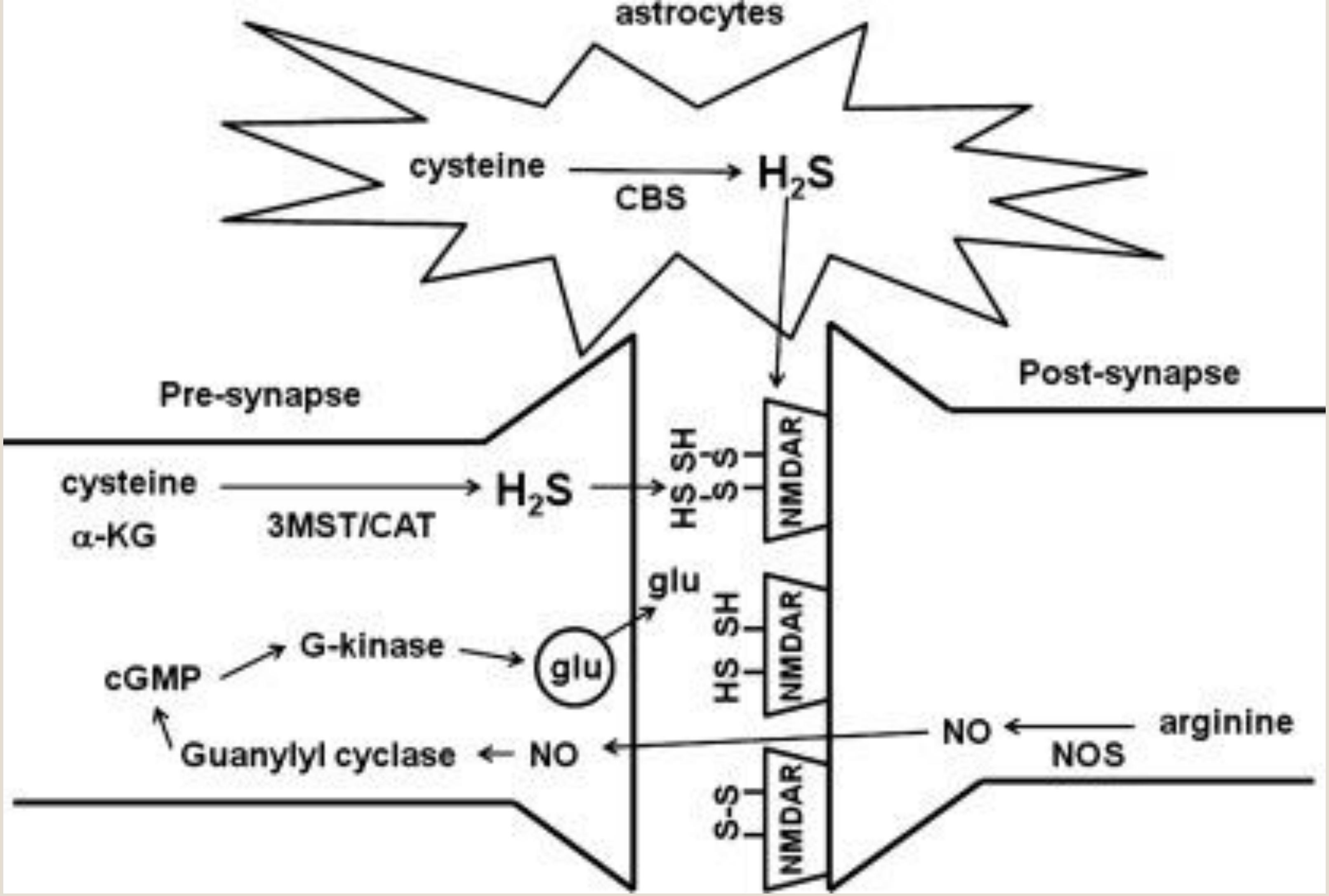




H_2S can be produced by three enzymes,
cystathionine β-synthase (CBS),
cystathionine γ-lyase (CSE), and
3-mercaptopyruvate sulfurtransferase (3MST), along with cysteine aminotransferase (CAT), which is identical to aspartate aminotransferase

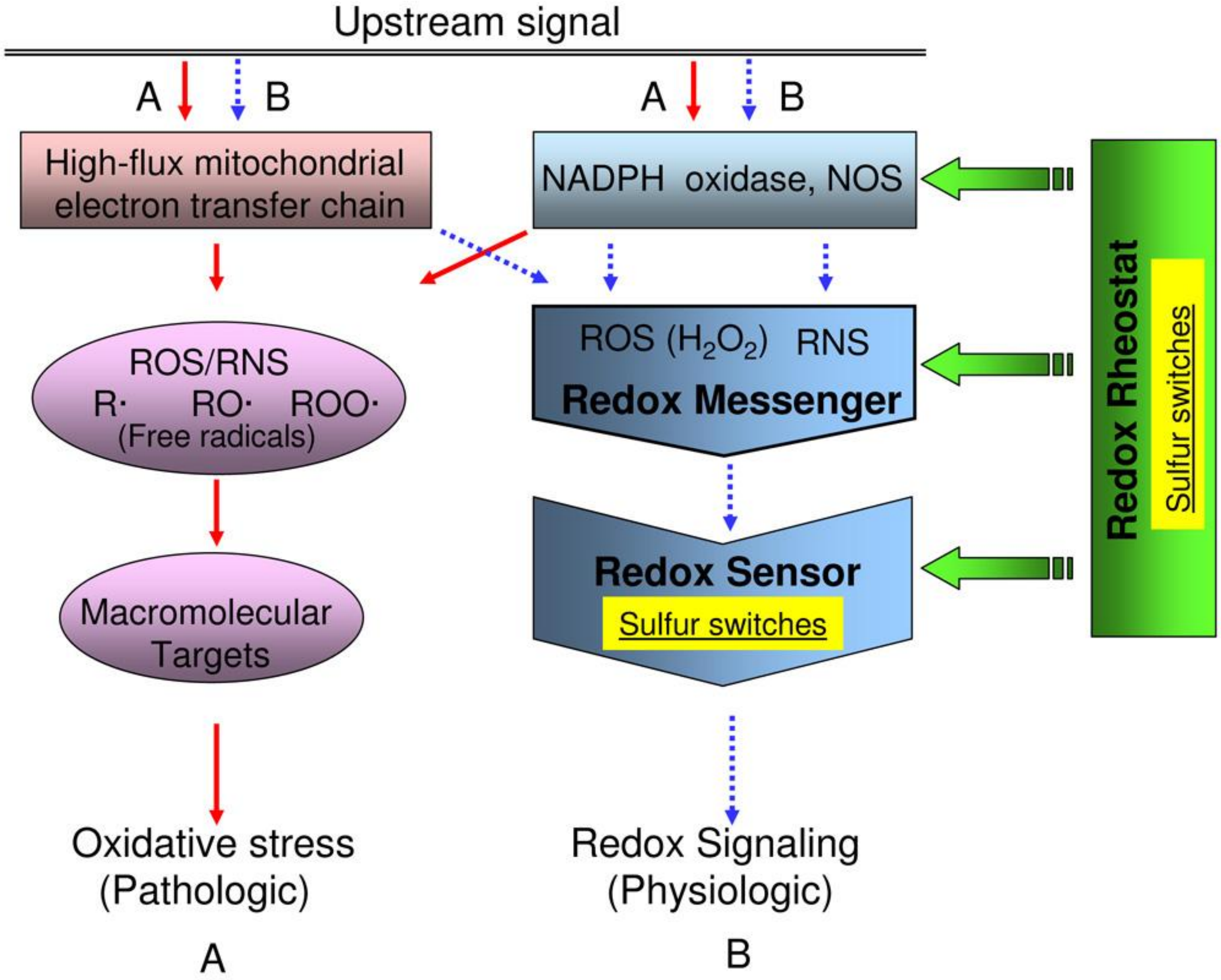


Modulation of enzyme activity by phosphorylation and sulfurhydration. The reversible incorporation of sulfur into proteins may modulate protein function as phosphorylation does on proteins. H_2S may directly induce sulfurhydration. Alternatively, H_2S may react with oxygen to form S^0 that adds to sulfhydryl groups of proteins.



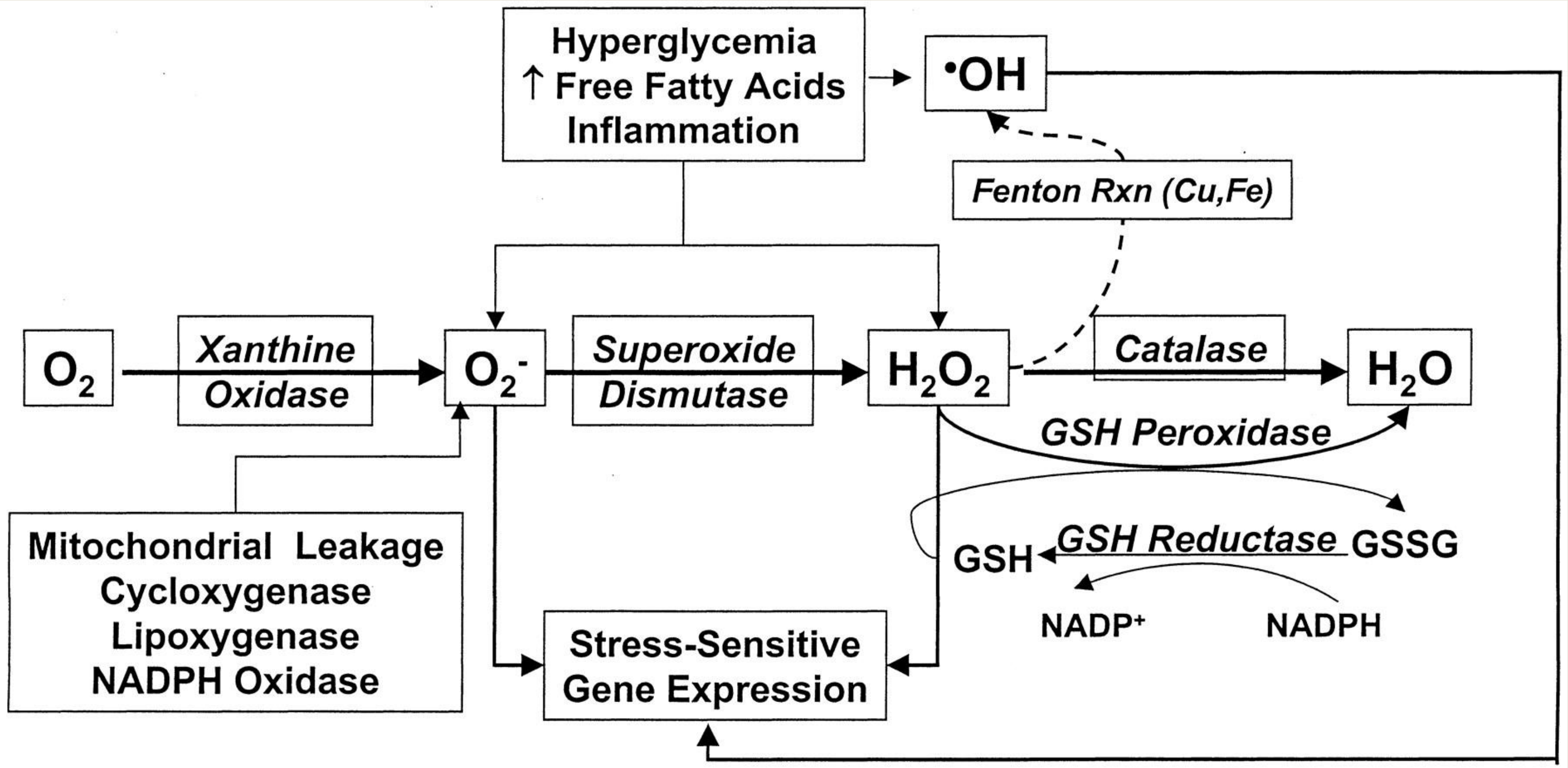
H_2S and nitric oxide (•NO) facilitate the induction of hippocampal long-term potentiation (LTP) with different mechanisms. •NO, which is produced from arginine, diffuses to presynapse to activate guanylyl cyclase, leading to the production of cyclic GMP that activates G-kinase to increase the release of a neurotransmitter glutamate. Although H_2S does not have any effect on guanylyl cyclase, it enhances the activity of NMDA receptors.

Redox signaling



Sulfur switches can exist as "Redox Sensors" and as "Redox Rheostats" in generic redox circuitry models. Simplified models show (A) oxidative stress pathway (solid line) associated with high levels of ROS, RNS, and free radicals from mitochondrial dysfunction, NADPH oxidase activation, and nitric oxide synthase activation triggered by pathologic upstream signals.
Redox signaling pathway (B, broken line) shows the process involving relatively low levels of diffusible reactive species associated with physiologic condition. In redox signaling, the small diffusible reactive species, termed "redox messengers" are generated by ROS/RNS sources and react with redox-sensitive macromolecules termed "redox sensors".

Exogenous and endogenous stimuli leading to ROS generation and activation of stress-sensitive gene expression.



Evans J L et al. Endocrine Reviews 2002;23:599-622

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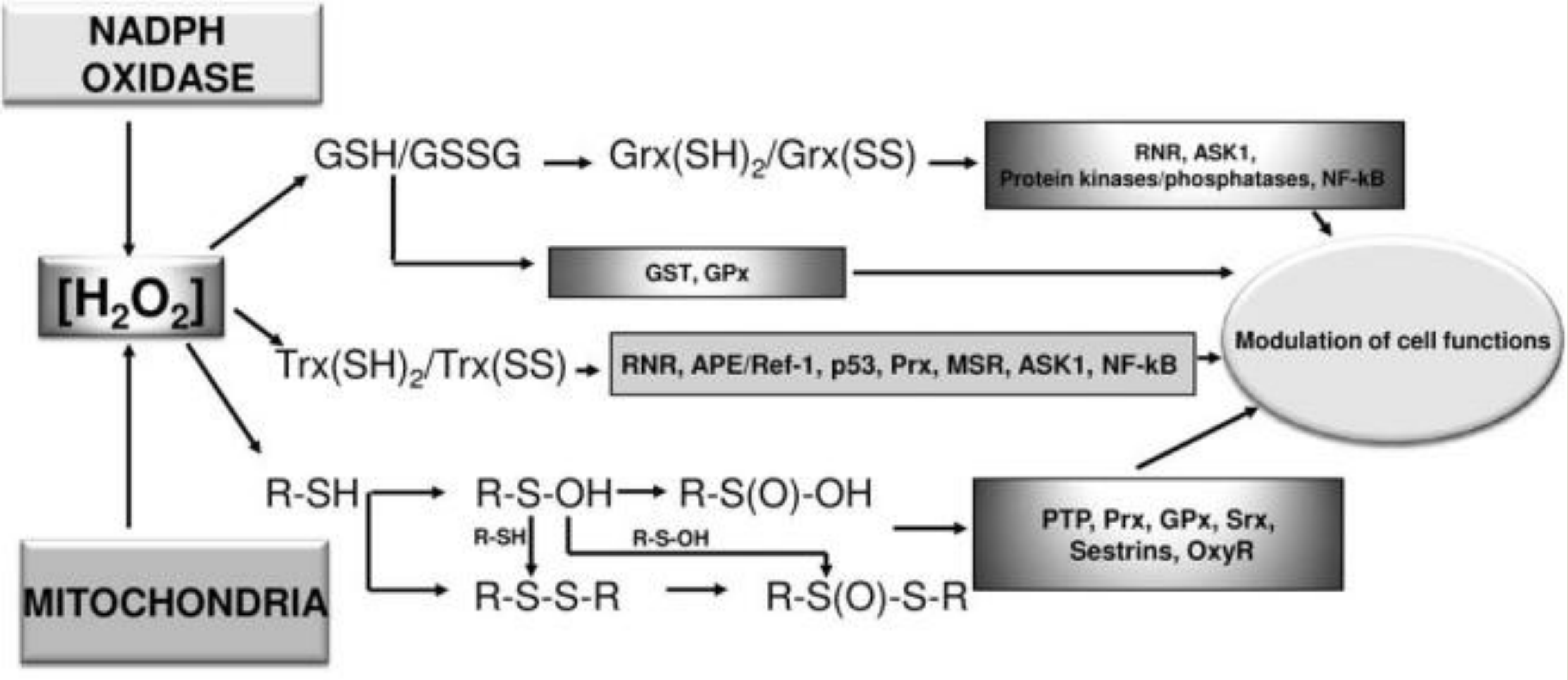
Signaling mediated by hydrogen peroxide



NADPH OXIDASE

$[H_2O_2]$

MITOCHONDRIA

GSH/GSSG → $Grx(SH)_2/Grx(SS)$ →

RNR, ASK1, Protein kinases/phosphatases, NF-kB

GST, GPx

$Trx(SH)_2/Trx(SS)$ →

RNR, APE/Ref-1, p53, Prx, MSR, ASK1, NF-kB

R-SH → R-S-OH → R-S(O)-OH

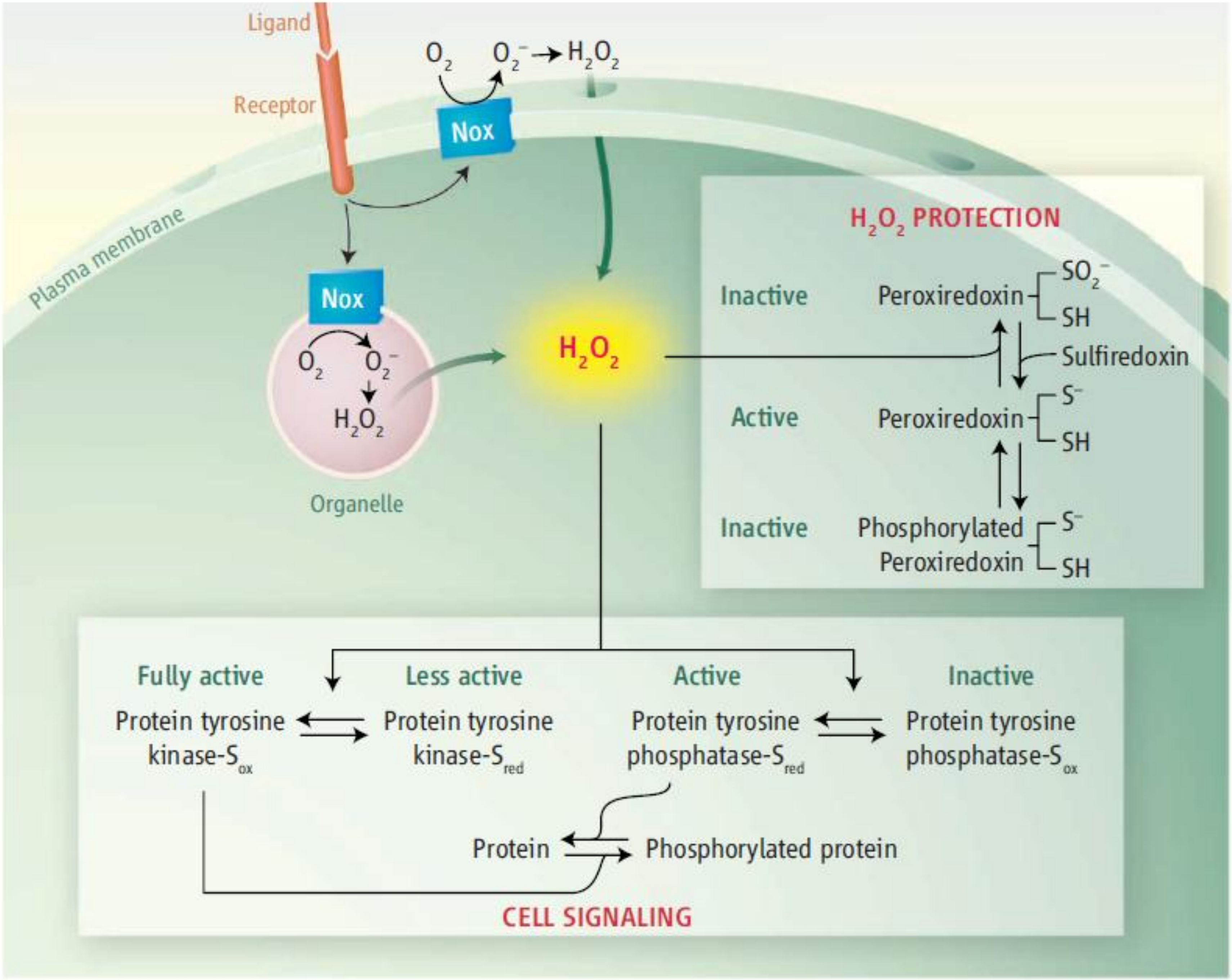
R-SH

R-S-OH

R-S-S-R → R-S(O)-S-R

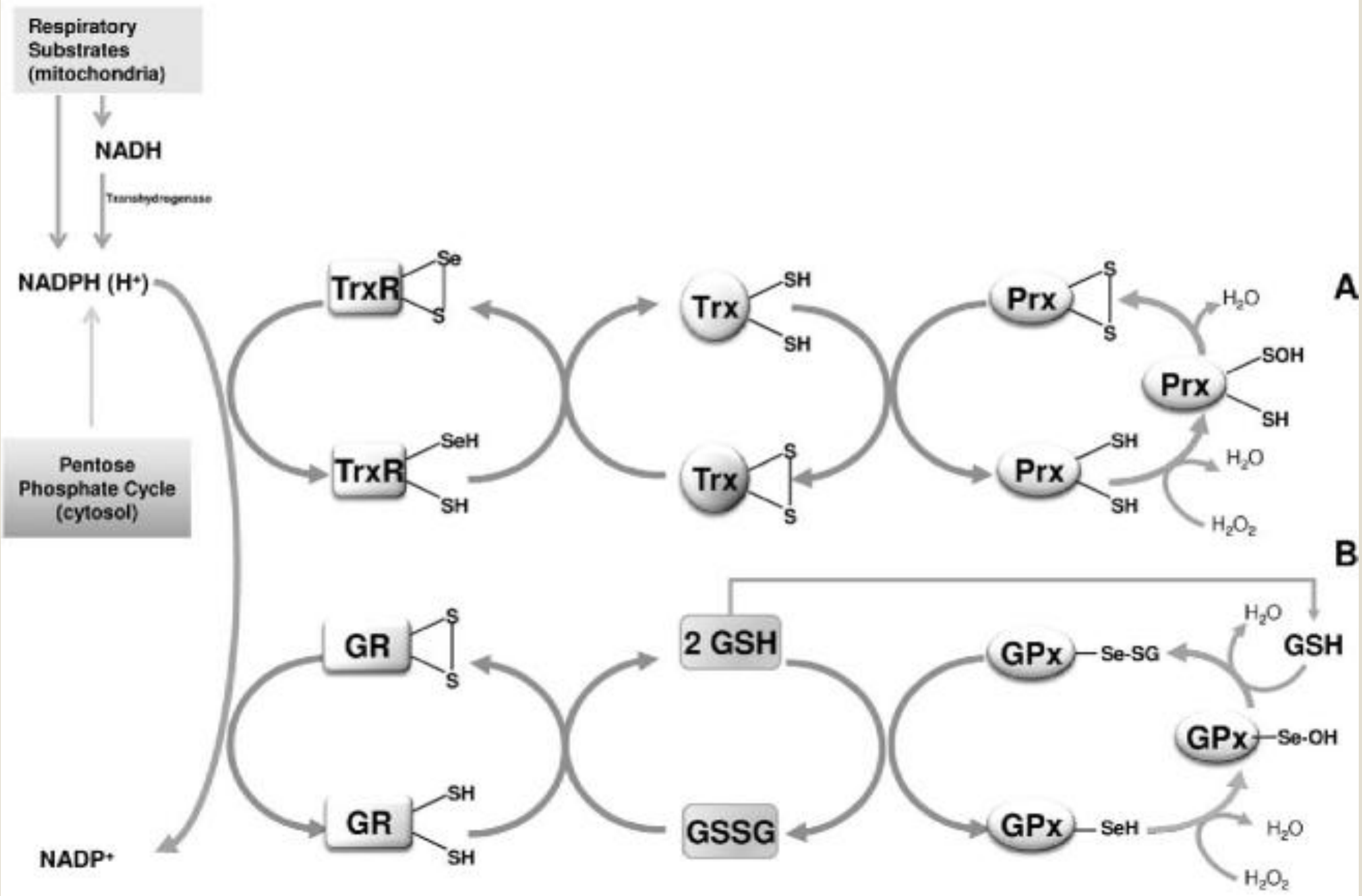
PTP, Prx, GPx, Srx, Sestrins, OxyR

Modulation of cell functions

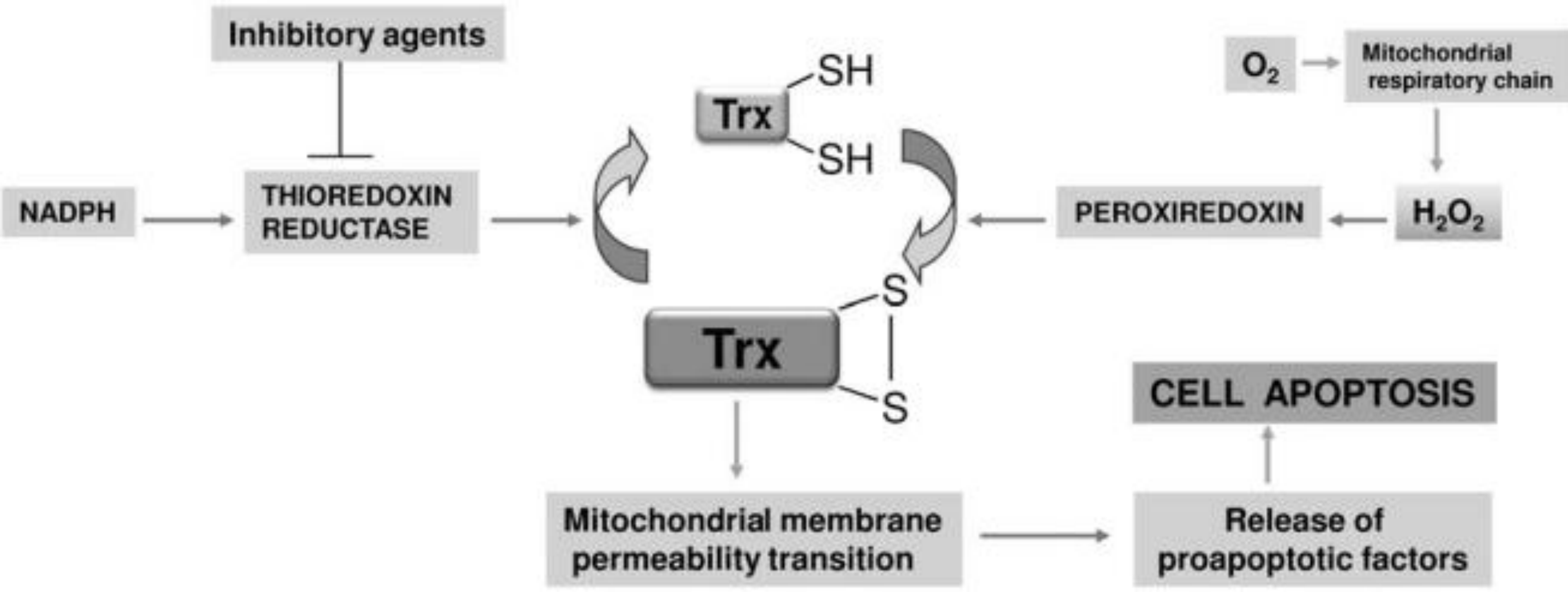


H_2O_2 production, protection, and signaling actions. Activation of various cell surface receptors activates Nox

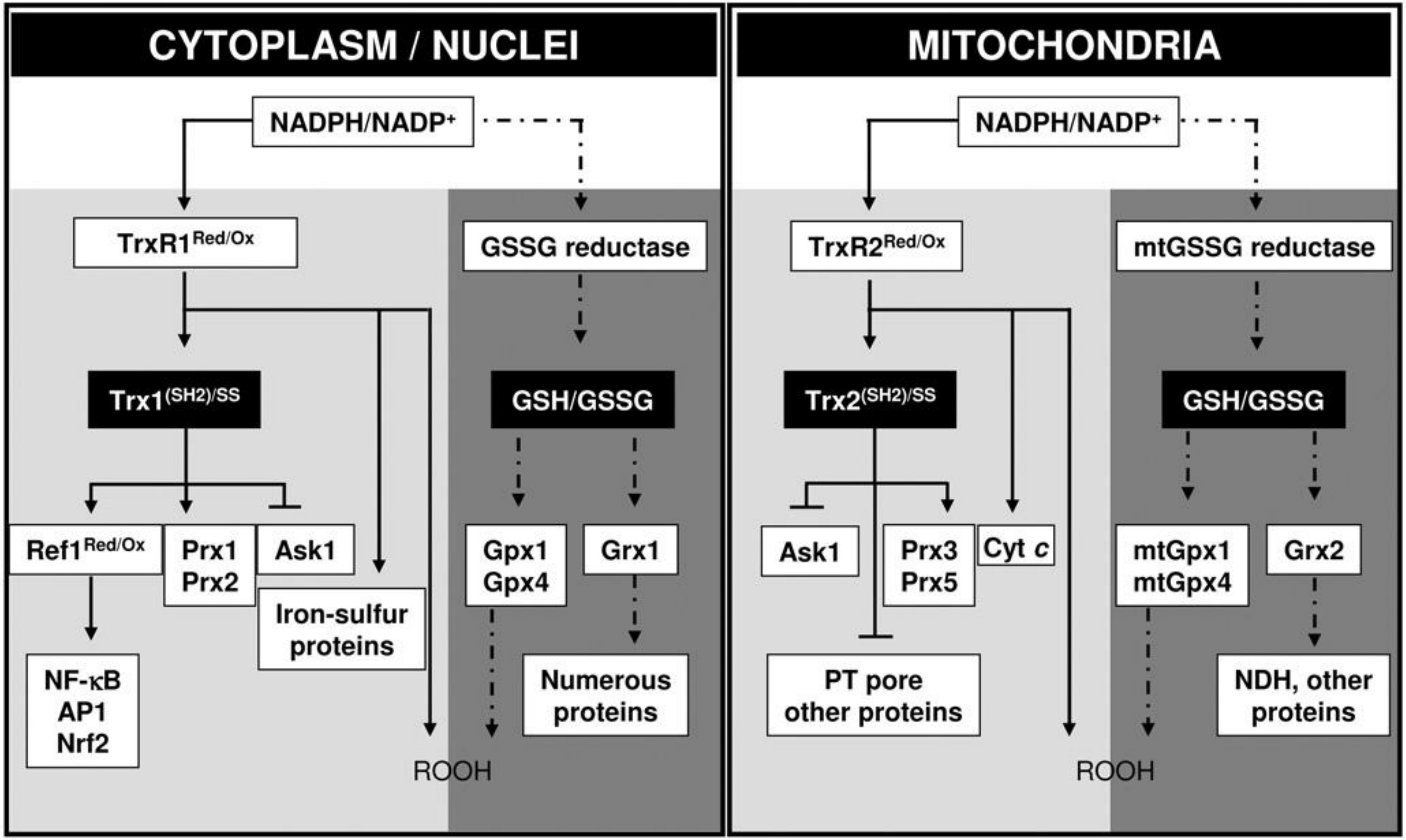
Reduction of hydrogen peroxide mediated by thioredoxin (A) and glutathione (B) pathways.



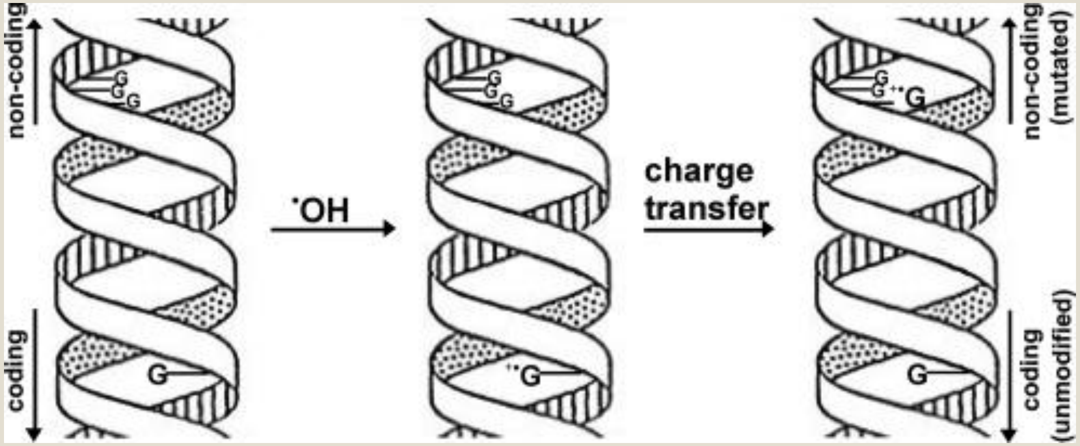
Mitochondrial alterations due to thioredoxin reductase inhibition

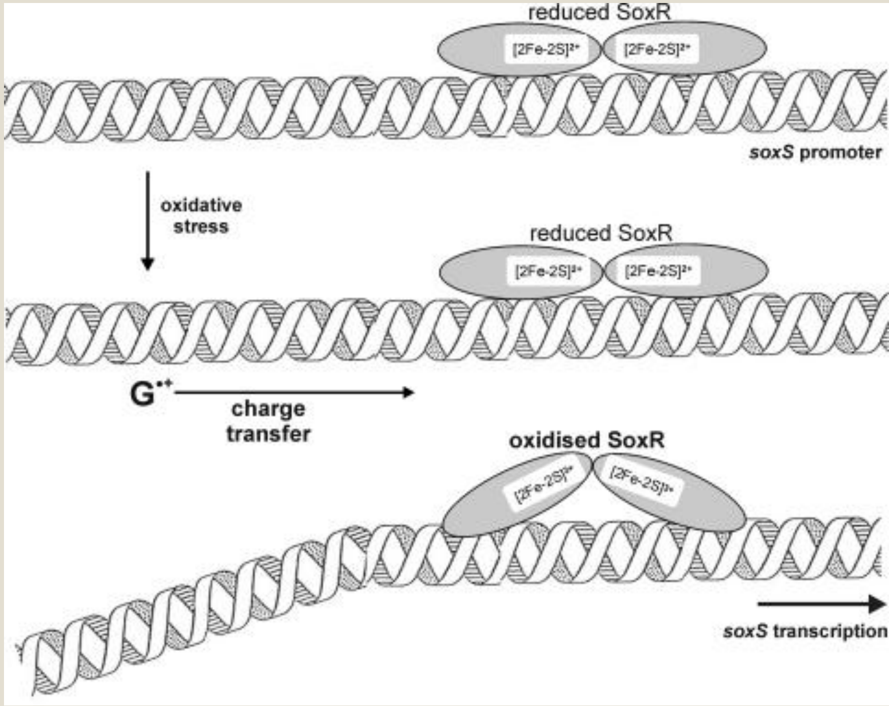

Inhibitory agents
NADPH
THIOREDOXIN REDUCTASE
Trx
SH
SH
O_2
Mitochondrial respiratory chain
PEROXIREDOXIN
H_2O_2
Trx
S
S
CELL APOPTOSIS
Mitochondrial membrane permeability transition
Release of proapoptotic factors

GSH and Trx-dependent systems support different protein-dependent systems in cytoplasm and nuclei (left), and in mitochondria (right).



Charge transfer in DNA





A model showing DNA-mediated activation of SoxR

Parameters of signal transduction

1. **Signal molecules**: properties (hydrophobicity) → location of R (surface/ intracellular)
2. **Properties of receptors** (R): specificity to signal (type of signal → type of R); ability to transduce the signal (binding/ catalytic); adaptation of R to signal (desensitization; feedback regulation)
3. **Signaling pathway**: structure of adaptors; amplification of signal (2 strategies depending on surface/intracellular location of R); integration of signal pathways (cross-talk; coordination of response)
4. **Molecular targets and time necessary for response** (quick/prolonged depending on protein synthesis)
5. For multicellular organisms: **hierarchy of signal systems**; location-, dose- and time-dependent effects

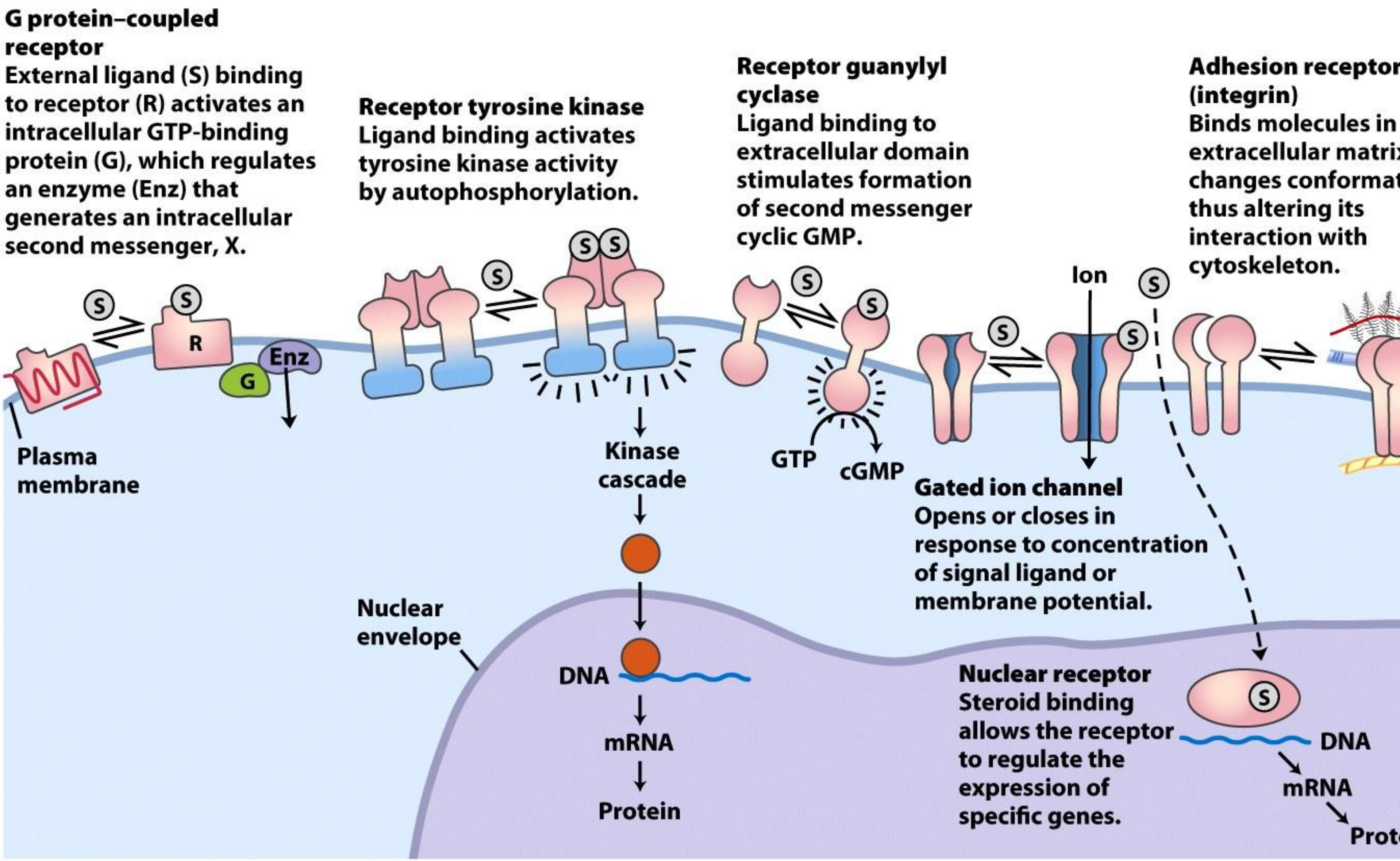
Outcomes of cell signaling

Enzyme activation

Amplification

Transcription and translation of specific genes

Inhibition, in some cases



G protein–coupled receptor
External ligand (S) binding to receptor (R) activates an intracellular GTP-binding protein (G), which regulates an enzyme (Enz) that generates an intracellular second messenger, X.

Receptor tyrosine kinase
Ligand binding activates tyrosine kinase activity by autophosphorylation.

Receptor guanylyl cyclase
Ligand binding to extracellular domain stimulates formation of second messenger cyclic GMP.

Adhesion receptor
(integrin)
Binds molecules in
extracellular matri
changes conforma
thus altering its
interaction with
cytoskeleton.

Gated ion channel
Opens or closes in response to concentration of signal ligand or membrane potential.

Nuclear receptor
Steroid binding allows the receptor to regulate the expression of specific genes.

Figure 12-2
Lehninger Principles of Biochemistry, Fifth Edition
© 2008 W. H. Freeman and Company

Parameters of signal transduction

1. **Signal molecules**: properties (hydrophobicity) → location of R (surface/ intracellular)
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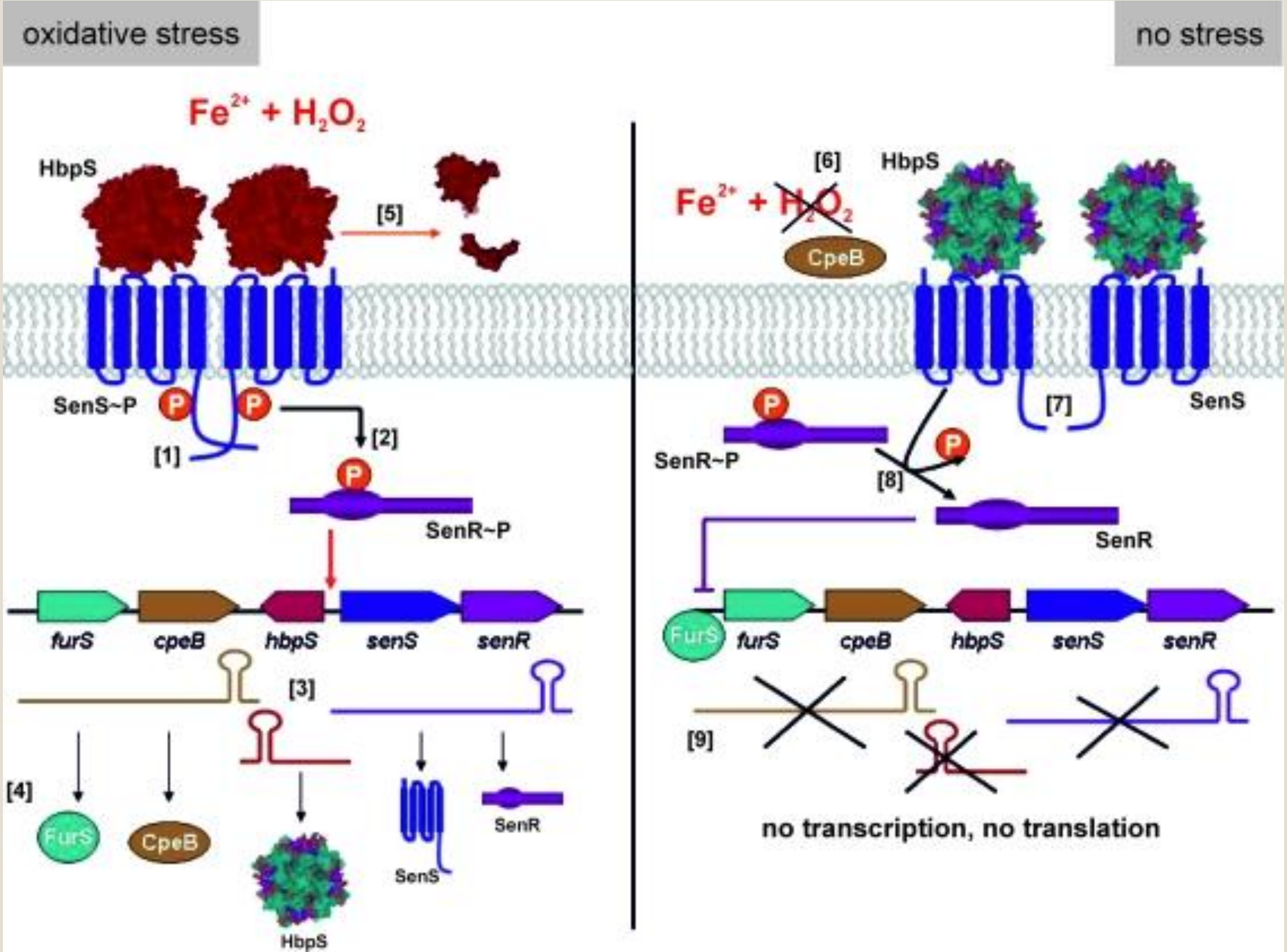
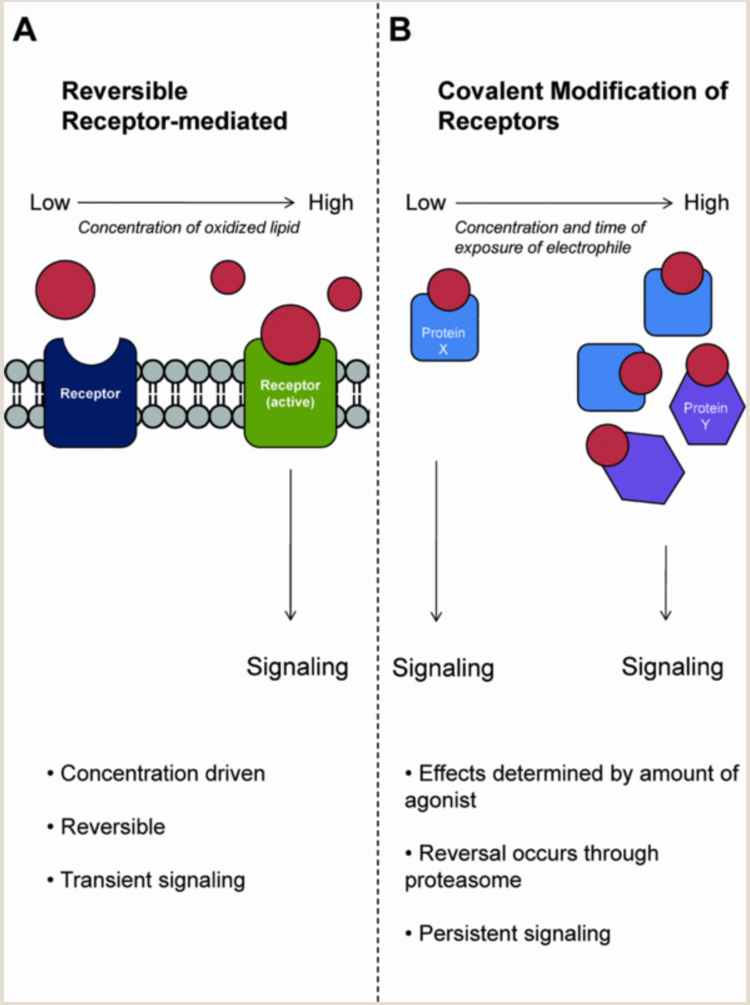
Schematic view of the mode of action of the redox-sensing system HbpS-SenS-SenR



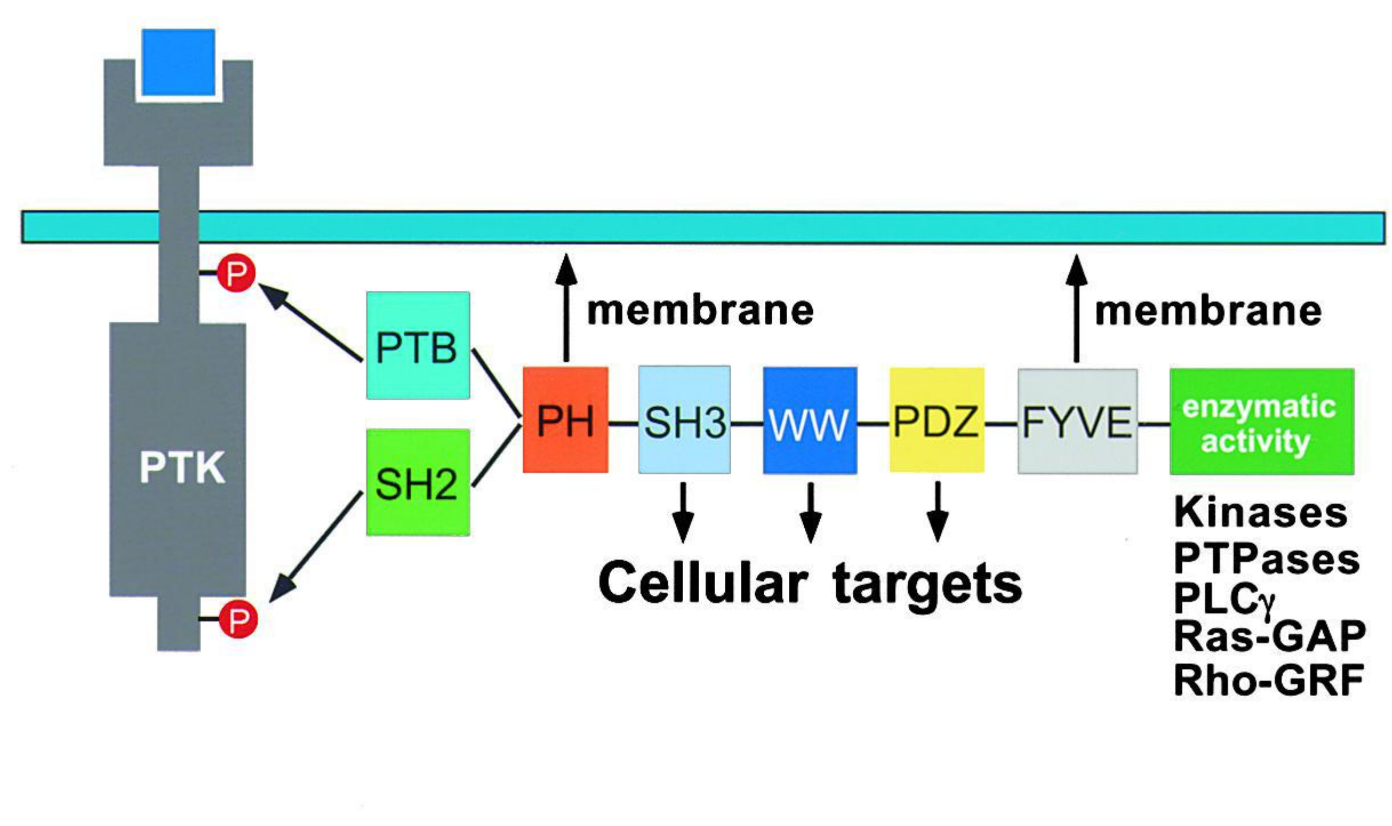
- Schematic view of the mode of action of the redox-sensing system HbpS-SenS-SenR. Under iron-mediated oxidative-stressing conditions (left) the oxidized and structure-changed form of HbpS (red) activates the autophosphorylation of the sensor kinase SenS, leading SenS∼P [1]. SenS∼P phosphorylates the response regulator SenR, leading SenR∼P [2]. This activates the transcription of hbpS and senS-senR, and de-represses the transcription of furS-cpeB [3]. Consequently, FurS, CpeB, HbpS, SenS, and SenR are produced [4] and participate in the antioxidative stress response. While SenS, CpeB, and HbpS are transported into the membrane (SenS) or extracellular space (CpeB and HbpS), FurS and SenR remain cytoplasmic. The oxidized and structure-changed form of HbpS undergoes a time-dependent degradation during oxidative stress [5]. Once CpeB associates with the mycelia, it degrades H2O2 providing S. reticuli with a nonoxidized environment and blocking the oxidation of HbpS [6] (right). Freshly synthesized and unmodified HbpS (mixed color, turquoise, and green) interacts with SenS and inhibits its autophosphorylation [7]. SenS in turn dephosphorylates SenR∼P, leading SenR [8]. This, together with FurS, represses the transcription of furS-cpeB and de-activates the transcription of hbpS and senS-senR [9].

Figure 2 Classical receptor-mediated signalling compared with signalling mediated through covalent modification


A
Reversible Receptor-mediated
Low
High
Concentration of oxidized lipid
Receptor
Receptor (active)
Signaling
• Concentration driven
• Reversible
• Transient signaling
B
Covalent Modification of Receptors
Low
High
Concentration and time of exposure of electrophile
Protein X
Protein Y
Signaling
Signaling
• Effects determined by amount of agonist
• Reversal occurs through proteasome
• Persistent signaling

Biochemical Journal 2012 442, 453-464 - Ashlee Higdon, Anne R. Diers and others.

Protein Signaling Modules (Domains)



SH2 and PTB bind to tyrosine phosphorylated sites
SH3 and WW bind to proline-rich sequences
PDZ domains bind to hydrophobic residues at the C-termini of target proteins
PH domains bind to different phosphoinositides
FYVE domains specifically bind to Pdtlns(3)P (phosphatidylinositol 3-phosphate)